Thrombocytopenia and neuropathy may lead to significant morbidity and mortality in patients with multiple myeloma (MM). In this study, we report the final results of a phase II study of bortezomib (V) in combination with doxil (D) for the treatment of patients with relapsed/refractory MM.

**Background**

Tumor microenvironment (ME) plays an important role in MM. It is associated with disease progression, metastases, and resistance to therapy. Targeting ME may be an effective way to overcome resistance in patients with MM.

**Methods**

We investigated clinically and experimentally whether bortezomib (V) in combination with doxil (D) in patients with hematologic malignancies may be an effective way to overcome resistance in patients with MM. We collected blood and bone marrow samples by means of flow cytometry. Plasma cells were identified as showing high-density expression of CD38 on the surface of plasma cells. CD11a (LFA-1), CD18 (Integrin αβ2), CD54 (ICAM1), and CD138 (syndecan-1) were also studied.

**Results**

In BM of PCL patients compared with the control, there were decreased RFIs of CD15 (15,2±1,6 vs 16,6±0,7) and CD25 (5,6±1,4 vs 10,4±0,8) and increased RFIs of CD3 (16,3±2,5 vs 10,1±0,7) and CD11a (15,4±1,5 vs 14,7±0,9). In BM of PCL patients, RFIs of CD29 (10,4±1,2) was lower than that occurring in control (11,6±0,9) while RFIs of CD3 (16,9±3,0 vs 14,8±1,3), CD54 (16,1±2,8 vs 12,3±0,3), CD11a (20,4±1,8 vs 18,3±0,8) was higher. BM leukemic cells with strong CD38 expression and CD138 expression showed antigen coexpression in following number of cases: CD54 in 16/19 (82%) tested, CD29 in 12/12 (100%), CD49d in 9/9 (100%), CD9 in 9/11 (82%), CD11a in 9/10 (90%), CD11b in 9/10 (90%), CD11c in 9/10 (90%), CD11d in 9/10 (90%), CD11e in 9/10 (90%), CD11f in 9/10 (90%) and CD11g in 9/10 (90%).

**Conclusion**

Bortezomib as a monotherapy may be an effective way to overcome resistance in patients with MM. We also investigated clinically and experimentally whether bortezomib (V) in combination with doxil (D) in patients with hematologic malignancies may be an effective way to overcome resistance in patients with MM.

**Acknowledgements**

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**References**


2. PCL patients showed a decreased relative number of BM: CD11a-cells (40±28% vs 72±10%), CD11b+cells (47±25% vs 88±7%), CD11c+/CD18-cells (42±27% vs 72±10%), CD44+cells (72±1,2% vs 98±4%), CD11b+cells (17±12% vs 55±10%) and PB: CD11a+cells (58±29% vs 96±3%), CD18+cells (58±29% vs 99±0,2%), CD11a+CD18+cells (58±29% vs 96±3%), CD44+cells (68±15% vs 98±0,9%).

3. BM of PCL patients compared with the control, there were decreased RFIs of CD15 (15,2±1,6 vs 16,6±0,7) and CD25 (5,6±1,4 vs 10,4±0,8) and increased RFIs of CD3 (16,3±2,5 vs 10,1±0,7) and CD11a (15,4±1,5 vs 14,7±0,9).

4. In BM of PCL patients, RFIs of CD29 (10,4±1,2) was lower than that occurring in control (11,6±0,9) while RFIs of CD3 (16,9±3,0 vs 14,8±1,3), CD54 (16,1±2,8 vs 12,3±0,3), CD11a (20,4±1,8 vs 18,3±0,8) was higher.
specific enzymatic assay. The localization of polyP in the myeloma cell lines was determined by confocal microscopy. The U266 myeloma cell line was used to study whether extracellular polyP affects Ig secretion and survival. Different human cell lines were used to test the specificity of polyP in viability. We analyzed Ig secretion of PC form Bone Marrow and Peripheral Blood after polyP addition. A conventional tetanus toxoid booster immunization was used to increase PC proportion in order to examine the apoptotic effects of polyP. Ig secretion and Apoptosis was determined by ELISA and FACS respectively. Results. Micromolar levels of polyP that is present principally as polymers of 75 phospho-phosphate units have been found in the U266 and IM9 myeloma cell lines. PolyP is accumulated in intracellular vacuoles similar to the previously reported platelet dense granules and acidocisomes of the unicellular eukaryotes. Addition of polyP to human PC produces an unexpected inhibition of Ig secretion and a stimulation of apoptosis. PolyP generates apoptosis specifically in PC, myeloma (malignant PC) cell lines, and B lymphoid cell lines. Normal B cells, T cells, total blood mononuclear cells, and non-lymphoid cell lines are not affected by polyP. In U266 myeloma cell line, polyP induces the externalization of phosphatidylserine, the activation of caspase-3, and the arrest of the cell cycle. Protec-tive effects of IL-6 do not overcome the polyP-induced apoptosis. Summary/conclusions. Taken together, our results and suggest for the first time the relevance of polyP for the humoral immune response and open prospects for polyP as a novel therapy for myeloma.

0755 METHYLATION STATUS OF THE P57KIP2 GENE IN PATIENTS WITH PLASMA CELL DYSCRASIAS

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Background. Oncogenesis is related to cell cycle deregulation. Abrupt DNA methylation, leading to silencing of regulatory genes, has emerged as one of the most frequent molecular changes in haematological malignancies. The p57KIP2 is a tumor suppressor gene that belongs to the CIP/KIP family of cyclin dependent kinase inhibitors that negatively regulate cell cycle progression. Aim. We have studied the methylation status of the promoter region of p57KIP2 gene in patients with multiple myeloma (MM) and Waldenström’s macroglobulinemia (WM) in order to correlate the methylation pattern with the disease’s phenotype. Patients and Methods. We have studied bone marrow and paired peripheral blood samples from 12 consecutive MM patients (9 male, 3 female, age range 50-83, median 59) and 2 consecutive WM patients (1 male and 1 female, age 75 and 47) years.

Figure 1. Localization of polyP on U266 and IM9 cells.

Samples from 9/12 MM patients and 2/2 WM patients were taken at diagnosis whereas the remaining 3/12 samples were taken during the course of the disease. Genomic DNA was extracted using standard protocols (Quiamp DNA mini kit). After bisulfite treatment procedure the DNA was PCR amplified with primers specific for the methylated and the unmethylated alleles of the gene. The PCR products were separated on 2% agarose gel. Bone marrow DNA from healthy donors served as negative control. We have also used human male genomic DNA universally methylated for all genes (Intergen Company, Purchase, NY) as positive control. Results. Two patients had stage IA disease and did not receive any treatment. For the treatment of MM patients had stage IIA or an unexpected disease and started on VAD chemotherapy, two patients were started on oral melphalan and methylprednisolone, one patient was on plateau, and two patients had progressive disease after having received VAD and were started on bortezomib therapy. One patient with WM was started on melphalan and prednisone and did not receive any treatment, and the other patient did not receive any treatment. Classical cytogenetic analysis was available on 5/12 MM and 1/2 WM patients and the karyotype was reported as normal. All patient samples showed no band corresponding to the unmethylated allele of the p57KIP2 gene. The band corresponding to the unmethylated allele was clearly visible in all samples. Conclusion. To our knowledge this is the first report on p57KIP2 methylation status in patients with plasma cell dyscrasias. Our data show that methylation of p57KIP2 gene is not a frequent event in the patients studied. Further studies are needed to confirm the above results.

0756 EVALUATION OF THE RELATION BETWEEN ANGIOTGENIC CYTOKINES, SELECTED BIOLOGICAL PARAMETERS AND PROGNOSTIC FACTORS IN MULTIPLE MYELOMA

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Background. Multiple myeloma is an unusually heterogenous disease with individually different course, response to therapy and prognosis. Up-to-date diagnostic and stratification systems have, however, an important limitation in their insufficient absorption of those parameters, that express intrinsic biological properties of myeloma cells and the microenvironment of the bone marrow. The aim of this study was to evaluate the relation of 10 biological parameters to 6 substantial prognostic factors in multiple myeloma. Methods. The analysed group consisted of 66 persons evaluated at the time of diagnosis, before the start of chemotherapy. For the assessment of serum levels of examined molecules were used following Methods. REA, RIA, ELISA and the technique of sandwich enzymatic immunoassay, for the assessment of proliferative and apoptotic properties were used propidium iodide (PC-PI) and annexin V (PC-PI) indices evaluated with the help of flow-cytometry. Statistical analysis was carried out using Pearson and Spearman tests for examining U-test according to Mann-Whitney. Results. High occurrence of abnormal serum level of evaluated parameter was found in the case of S-β2-microglobulin (95,5%), S-thymidinekinase (57,5%), S-sVCAM-1 (78,5%), S-sITCP (87,0%), S-soluble osteoprotegerin (sOPG 76,5%), S-Syndecan-1 (56,5%) and low index of apoptosis of plasma cells (PC-PI, 78%). Correlation analysis (Pearson test) revealed a mutual relationship between serum levels of B-2-microglobulin to sVCAM-1 (r=0,39, p=0,002), sICAM-1 (r=0,33, p=0,011), sOPG (r=0,53, p=0,001), SHGF (r=0,34, p=0,006), Syndecan-1 (r=0,38, p=0,003) and sFas (r=0,42, p=0,001); of S-albumin to sVCAM-1 (r=-0,29, p=0,036), ICTP (r=-0,33, p=0,016), sOPG (r=-0,63, p=0,000), SHGF (r=-0,39, p=0,003) and SYndecan-1 (r=-0,29, p=0,042) of S-thymidinekinase to Syndecan-1 (r=-0,46, p=0,000) and sFas (r=-0,29, p=0,019). In neither of the cases was found the relation of PINP and VEGF to any of the evaluated prognostic factors. There was no relation found between any of the analysed parameters and PC-PI or PC-PI. With the use of U-test there was found a relationship of serum levels of sIL-6R (< > 100IU/l) to B-2-microglobulin (r=0,01), albumin (p=0,002) and to PC-PI (p=0,046). Conclusion. The above study established the possibility to enrich the traditional algorithms used in clinical practice for individual characteristics of MM with the parameters sOPG, SHGF, Syndecan-1 and sFas. Founded by MSM 6198959205.
evaluation of serum levels of assessed parameters were used following Methods. radioenzymatic assay (thymidinekinase), radioimmunoanaly-sis (β-2-microglobulin, ICTP, PINP), method of enzymoimmunoassay (sIL-6R, sVCAM-1, sICAM-1, sOPG and sRANKL) and the technique of quantitative sandwich enzymatic immunoassay (sHGF, sVEGF, bFGF, syndecan-1/CD138 and sfas). Statistical analysis was carried out using Pearson’s χ² test. Comparision of the parameters U test according to Mann-Whitney (p<0.05). Results. Statistically significant differences were found out between MGUS and MM in case of comparison of serum levels of sIL-6R (p<0.02), ICTP (p<0.001), sHGF (p<0.001) and syndecan-1/CD138 (p<0.001), whereas in case of sVCAM-1, sICAM-1, PINP, sOPG, sVEGF and sFas there were no statistically significant dif-ferences. Within the analysis of the frequency of the occurrence of abnor-mal values in the MM and MGUS group there were significant differ-ences not only in the case of standard parameters such as β-2-microglobulin, thymidinekinase creatine and albumin, but also in the case of sIL-6R, ICTP, sHGF, and syndecan-1, however not in the case of compari-sion of the values of sVCAM-1, sICAM-1, PINP, sOPG, sVEGF and sFas. Measurement of serum levels of sRANKL and soluble form of bFGF was of no avail due to very low values of these parameters. Conclusion: The analysis of the 10 parameters, that are altogether very close related to the biological properties of clonal plasma cells or to the changes of bone marrow microenvironment revealed from the point of the contribution for the MM of MGUS from clinical point of view that the only significant parameters were the only serum levels of sIL-6R, ICTP, sHGF and syndecan-1 (sCD138), i.e. the parameters with certified significance for the MM prognosis evaluation.

**0758**

**RELATIONSHIP OF SERUM FREE LIGHT CHAIN LEVELS TO DEGREE OF MULTIPLE MYELOMA PROGRESSION**

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**Background**

Multiple myeloma is a malignant disease characterised by clonal proliferation and accumulation of neoplastically transformed B-line elements, producing monoclonal immunoglobulin (MIG) demonstrable in serum and/or urine. Plasma cells also produce free light chains (FLC) κ and λ, that are not fixed in MIG molecule. Aim of the study was a comparison of serum FLC levels and κ/λ (κ/λ) ratio between stages of Durie-Salmon (D-S), International Prognostic Index (IPI) and South West Oncology Group (SWOG) staging systems. Methods. Prospective study includ-ed 145 patients with multiple myeloma, examined during one year period. Serum FLC levels were assessed using FREE-LITE Immunotech methods, values of β2-microglobulin were obtained by RIA. Mann - Whitney’s U-test was used for statistical evaluation. Results. Abnormal values of serum FLC and κ/λ ratio were assessed in 79% and 81%, κ secretion was present in 67%, λ in 33%. Comparing with each stage of D-S staging system, significant levels of dominant chain (p<0.005) and κ/λ ratio (p<0.005) were found between stages II and III in κ group only. Differences in values between other stages were not significant. Comparing subgroup A and B (serum creatinine over 177 µmol/L), significant differences were found in levels of dominant and alternative chain in κ group (p<0.047 and p<0.014) and also in λ group (p<0.007 and p=0.046), but there was no significant difference between κ/λ ratio values. Using IPI staging system, significantly different levels of dominant kappa chain were found in κ group between stages I and II (p=0.029), between stages I and III in values of κ chain (p=0.029) and κ/λ ratio (p=0.04). In lambda group also were found differences in λ dominant chain and κ/λ ratios between stages I and II (p=0.011) and also between stages I and III (p=0.0001 and p=0.0013). Differences of FLC values between stages II and III in both groups were not significant. In case of SWOG staging system, in κ group, differences in levels of dominant chain between stages I and II (p=0.038) and I and III+IV (p=0.085) were as-sessed. In λ group were found different values of dominant chain inκ/λ ratio between stages I and II (p=0.029 and p=0.042) and also between I and III+IV (p=0.002 and p=0.004). Between stages II and III+IV was found a difference in dominant chain in lambda group only (p=0.047). Conclusions. Disease progression degree evaluated using dynamic markers - serum albumine and β2-microglobulin (IPI and SWOG system), correlate with serum FLC levels more expressively, than traditional Durie-Salmon staging system. Serum FLC levels depend on kidney function, but κ/λ ratio values are not affected by impaired renal function.

**0759**

**VEGF EXPRESSION AND MICROVESSEL DENSITY IN PATIENTS WITH MULTIPLE MYELOMA: CLINICAL AND PROGNOSTIC SIGNIFICANCE**

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**Background**

Angiogenesis or new vessel formation is an essential component in the growth and progression of solid malignancy. However, conflicting data are reported on clinical significance of VEGF deregulation and microvessel density (MVD) in multiple myeloma (MM). Aim: To analyse the impact of the study population. Analyze the incidence of VEGF expression and grade of MVD, and to correlate these findings with pathohistological and clinical features of newly diagnosed myeloma patients. Patients and methods. We analyzed bone marrow biopsy specimens obtained from 59 patients with MM. The clinical staging was done according to the Durie and Salmon classification (four patients had disease stage I, 15 patients stage II and 39 patients stage III). Expression of VEGF and MVD were analyzed using standard immunohistochimical analysis of B5-fixed and routinely processed, paraffin-embedded bone marrow specimens with antibodies against VEGF and CD34, respec-tively. Median MVD was estimated in three hot spots at magnification x400, according to the method of Weidner et al. VEGF immunoactivity was estimated on the basis of intensity and percentage of positive plasma cells. Results. VEGF was expressed in 47 out of 59 (79.66%) specimens. No statistical correlation could be found between VEGF overexpression and age, clinical stage, degree of osteolytic lesions, types of monoclonal protein, hemoglobin concentration, platelet count, serum concentration of creatinin, calcium and albumins, the extent of bone marrow infiltration, histological grade and proliferative activity (measured with Ki-67 immunoactivity). In addition, no significant difference regarding overall survival was found between VEGF positive and VEGF negative cases (29 months vs. 34 months, p=0.5). Median MVD was 15 (range: 1-89). We found significant correlation between MVD and histological grade, the extent of bone marrow infiltration and proliferative activity. Although MVD showed prognostic impact on overall survival in univariate analysis (p=0.009), multivariate analysis identified only age, hemoglobin concentration and proliferative activity as independent prognostic factors. Conclusions. The upregulated VEGF is seen in plasma cells in the majority of myeloma cases. However, the relationship between this finding and pathogenesis of the disease still remains to be established. The microvessel density can predict poor survival in myeloma and be helpful in choosing optimal therapeutic modality in every individual patient.

**0760**

**CLONOCENIG CAPACITY OF BONE MARROW CELLS IN PATIENTS WITH MULTIPLE MYELOMA: THE INFLUENCE OF ARSENIC TRIOXIDE AND BORTEZOMIB ON THE PROLIFERATION OF CFU-F AND CFU-GM**

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Arsenic trioxide (As2O3) and bortezomib were tested as therapeutic agents for a variety of malignancies. The aim of our study was to investigate in vitro effects of As2O3 and bortezomib on clonogenic capacity of haematopoietic and mesenchymal progenitor cells in patients, newly diagnosed multiple myeloma and patients with multiple myeloma resistant to standard chemotherapy. Materials and methods. Bone marrow samples were obtained from 24 patients with multiple myeloma: 10 before treatment and 14 patients resistant to standard chemotherapy, 11 females and 13 males, 16 with IgG, 6 with IgA, 1 with IgD, 1 with IgM. Mononuclear cells (MNC) were cultured without As2O3 or bortezomib and with As2O3 at a concentration of 0.2 mmol/l and bortezomib at a concentration of 10 and 20 ng/ml. MNC were plated in a standardized methylcellulose medium (MethodCult 4434, StemCell Technologies and MesenCult (StemCell Technologies). Colony formation of haematopoietic progenitors (CFU-GM and BCU-E) and mesenchymal progenitor cells (CFU-F) were based on colony counting of the colonies were assessed on day 14 of cultures. CFU-GM, BCF-E and CFU-F expressed as the percentage of decrease versus control and the mean and standard deviation (SD) of colony inhibition for each concentration of As2O3 or bortezomib were calculated across all samples. Results. In all patients with resistant myeloma and 2/3 of newly diagnosed patients we
observed an increased number of mesenchymal progenitors in cultures. As-O and bortezomib caused reduction of CFU-GM and BFU-E formation after 14 days of incubation to 1% and 0.5% of control values respectively. Formation of CFU-F was completely inhibited by As-O and bortezomib. Conclusions. Our data clearly demonstrate that in in vitro conditions exposure to As-O or bortezomib even in low concentration is able to induce growth inhibition of haematopoietic progenitor cells in patient plasma cells. Bortezomib, and As-O inhibit completely formation of mesenchymal progenitor cells in this group of patients. A combination of direct toxicity against leukemic cells with proapoptotic activity of bortezomib or As-O may be the optimal characteristic of a successful antimyeloma agent in particular in patients with increased number of CFU-F before treatment.

**0761**
CHARACTERIZATION OF THE PLASMA CELLS OF MULTIPLE MYELOMA BY SERIAL ANALYSIS OF GENE EXPRESSION


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**Backgrounds.** In the last years, several large-scale gene expression studies with array based hybridisation have been performed in multiple myeloma. Numerous genes correlated with the disease have been identified. However, the molecular mechanisms involved in the disease are still not completely elucidated. More recently, the serial analysis of gene expression (SAGE) method has allowed the global analysis of genes expressed in a determined cell or tissue. However, to the best of our knowledge, no consistent studies in plasma cells of MM have already been performed using the SAGE method. Aim. The aim of this study was to characterize the plasma cells of MM by SAGE. Methods. Purified normal plasma cells (PNPC) differentiated from bone marrow B cells of a healthy individual and purified neoplasic plasma cells from a newly diagnosed MM patient were obtained by magnetic sorting in a column, using the CD-138 antibody Macs microbeads (MACS, Miltenyi Biotec, Germany). Both SAGE libraries, PNPC and MM, were obtained using the I-SAGE Kit (Invitrogen, Life Technologies, USA), in accord with the manufacturer procedures. The expression of a group of genes arbitrarily selected were further investigated by quantitative polymerase chain reaction real-time (qRT-PCR) in the sample of SAGE MM and in other samples of MM patients, in comparison with SAGE PNPC sample, with the purpose of verify the reliability of the results obtained by SAGE. The functional classification of genes was performed according to the Gene Ontology Consortium. Results. We generated, after automatic sequencing, a total of 64,965 tags from the MM plasma cells and 77,000 tags from the normal plasma cells, representing 24,601 and 25,527 unique tags, respectively. In the comparison of both profiles, 476 differentially expressed transcripts were identified (p<0.01; fold 5). Approximately 70% of the unique tags from both profiles were known genes or annotated sequences, and 30% corresponded to tags that may represent novel transcripts. The expression of 8 up-regulated genes (CCND1, DUSP1, FOS-B, IGHG3, IGKC, V-FOS, V-JUN, PRDM2), 6 down-regulated genes (CD19, CD40, EFHand, FCER2, IL6-ST e RNAse1) and two normally expressed genes (B2M e XBP-1) on MM library were evaluated by qRT-PCR in the SAGE MM and in other samples of MM patients. Similar mean expression values were found in both materials. A distinct mean expression value of the PRDM2 gene (109.51 in MM library vs 1.00 in MM samples) was the unique discordant result. The functional classification of genes revealed abnormal expression of genes involved in transcription, signalling, cell proliferation and apoptosis. Conclusions. We identified abnormal expression of genes involved in fundamental processes of plasma cells proliferation and survival, which may contribute to the comprehension of MM pathophysiology, and to the identification of new targets for MM therapy.

**0763**
RENAI IMPAIRMENT IN MULTIPLE MYELOMA PATIENTS FOLLOWING ZOLEDRONIC ACID OR IBANDRONATE TREATMENT

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**Backgrounds.** Although bisphosphonates prevent skeletal complications, agents differ with respect to renal safety. Ibandronate (IB) is a single-nitrogen, noncyclic bisphosphonate that has shown a renal safety profile comparable to placebo in phase III trials. This retrospective study aimed to compare renal impairment rates in multiple myeloma (MM) patients treated with IB or zoledronic acid (ZO). Methods. Medical records in a German oncology clinic from May 2001 to December 2005 were retrospectively reviewed. Creatinine measurements were analyzed from baseline (before ZO or IB treatment) to last evaluation for each patient. Renal impairment was defined as (1) a serum creatinine (Scr) increase of ≥0.5 mg/dl or ≥2.0 mg/dl from baseline values of <1.4 mg/dl or <2.6 mg/dl, respectively, or (2) a ≥25% decrease in glomerular filtration rate (GFR, abbreviated MDRD formula) from baseline. Patients treated sequentially with both ZO and IB were included as separate observations. Multivariate analyses were conducted using the Cox proportional hazards model and the Andersen-Gill (A-G) extension of the Cox model for multiple-events. Results. ZO 26 patients, 69 received ZO and 40 received IB, with 25 patients receiving both drugs. Compared with IB, the ZO group had a significantly better baseline renal function (mean Scr 1.01 vs 1.84, p=0.006; mean GFR 75.9 vs 57.3, p=0.0002). Data analysis showed that ZO treatment increased the relative risk (RR) of renal impairment by ~3-fold compared with IB (renal impairment rates: ZO 57.7% vs IB 14.4%, RR=2.3, p=0.0001; GFR 23.4%, RR=2.6, p=0.0002 [GFR]). The incidence rate of renal impairment was higher for ZO than IB (Scr: 1.03 vs 24.30%, RR=2.6, p=0.0002 

**0762**
THE PREVALENCE OF K-RAS AND N-RAS MUTATIONS IN BRAZILIAN PATIENTS WITH MULTIPLE MYELOMA

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**Background.** Point mutations affecting codons 12, 13 (exon 1) and 61 (exon 2) of the N- RAS gene and codons 12, 13 (exon 1) of the K-RAS gene are identified in about 30.0% and 10.0% of MM patients of the Northern hemisphere, respectively. Aim. Since there are no reports about the prevalence of RAS genes mutations in MM Brazilian patients, this was the aim of the present study. Methods. DNA from bone marrow aspirates of 252 patients with MM were investigated for whole exons 1 and 2 of the N-RAS gene and whole exon 1 of the K-RAS gene by direct sequencing of DNA amplified by real time polymerase chain reaction. Results. Three out of 252 (2.08%) MM patients presented RAS mutations. Heterozygous mutations of the N-RAS gene were found in seven out of 252 (2.78%) patients. Three of them (1.19%) presented a mutation in exon 1, at codon 4 (TAC_AAC), one patient (0.40%) presented a mutation in exon 1, at codon 10 (GGA_GAA), and 2 patients (0.79%) presented a mutation in exon 2, at codon 61 (CAA_CAT). A mutation in exon 2, at codon 65 (AGT_ACT) was identified in one patient (0.40%). Heterozygous mutations of the K-RAS gene at codons 7, 12 and 13 were found in 46 out of 252 (18.25%) patients. Twenty-six of them (10.31%) presented a new mutation at codon 7 (GTG_GCC). One patient (0.40%) presented a mutation at codon 12 (GGT_GTT). A mutation at codon 13 (GCC_GCC) was identified in 19 patients (7.54%). Similar frequencies of the K-RAS gene mutation at codon 7 were observed in patients stratified by age (46.15% in patients ≥60 years vs 53.85% in patients >60 years; p=0.83), gender (57.70% in males vs 42.30% in females; p=0.80), ethnic origin (9.61% in blacks vs 17.69% in caucasian; p=0.24), status (73.08% in patients at diagnosis vs 26.92% in patients at progression of the disease; p=1.00) and stage of the disease (30.77% in patients at stages I+II vs 69.23% in patients at stage III; p=0.02). There were no difference between the median percentages of the plasma cells obtained from the bone marrow of patients with and without K-RAS gene mutation at codon 7 (26.26% vs 26.20%, p=0.70). Summary. The functional classification of genes or annotated sequences, and 30% corresponded to tags that may represent novel transcripts. The expression of 8 up-regulated genes (CCND1, DUSP1, FOS-B, IGHG3, IGKC, V-FOS, V-JUN, PRDM2) and two normally expressed genes (B2M e XBP-1) on MM library were evaluated by qRT-PCR in the SAGE MM and in other samples of MM patients. Similar mean expression values were found in both materials. A distinct mean expression value of the PRDM2 gene (109.51 in MM library vs 1.00 in MM samples) was the unique discordant result. The functional classification of genes revealed abnormal expression of genes involved in transcription, signalling, cell proliferation and apoptosis. Conclusions. We identified abnormal expression of genes involved in fundamental processes of plasma cells proliferation and survival, which may contribute to the comprehension of MM pathophysiology, and to the identification of new targets for MM therapy.

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patients who switched from ZO treatment had a significantly higher risk of renal impairment than IB monotherapy patients (renal impairment rates: switchers 40.9% vs monotherapy 6.7%, RR=6.1, p=0.023 [SCr]; 63.6% vs 26.7%, RR=2.4, p=0.029 [GFR]) but experienced a significant trend towards improved renal function during the IB treatment period after a significant trend towards renal deterioration in the ZO treatment period. Multivariate analysis using the Cox proportional hazards model and the A-G model for multiple-event analysis consistently found significantly higher hazards ratios for ZO over IB, after adjusting for differences in characteristics between the two treatment groups. (SCr: Cox=4.2, p=0.016; A-G=0.0, p<0.0001; GFR: Cox=4.2, p=0.001; A-G=3.6, p<0.0001). Conclusions. In this retrospective review, MM patients were significantly more likely to experience renal impairment with ZO than with IB. Among IB patients, those previously treated with ZO had a higher risk of renal impairment than monotherapy patients. A prospective randomized study is warranted for further validation.

**0765**

ANALYSIS OF RESPONSE AND FOLLOW-UP IN RELAPSED REFRACTORY MYELOMA RECEIVING BORTEZOMIB


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**Background.** Bortezomib, a boronic acid dipeptide a novel targeted therapy is a proteasome inhibitor that has been shown to be effective in the therapy of multiple myeloma (MM). Aims. To evaluate the efficacy of Bortezomib+Dexa or Melphalan in patients with refractory relapsed MM treated in our Department from December 2003 to January 2008. Patients and Methods. We have included 34 patients with relapsed MM that had been treated with one or more lines of therapy, (VBCP/VBAB, VAD, PBSCT one or tandem, TACYDEX, radiotherapy). Bortezomib (1,3 mg/m² twice weekly two weeks on days 1,4,8,11 in a 21-day cycle) in an outstanding regime were administrated. The response was evaluated according to the criteria of the International Myeloma Working Group and Southwest Oncology Group Foundation 2003. CR: without symptoms, no monoclonal component (MC) detected by immunofixation electrophoresis (IFE) (Sebia standardized procedure), PR: reduction of MC >50%, Minimal Response(MR): reduction of MC 25-50%, Clinical response (Clin R): no clinical symptoms, and non-response(NR). Adverse effects were registered. Results. 34 patients (males 41.7%), mean age 60 years (35-74), over 65 years (50%). Type IgG: 11 (6 II-A, 5 III-A); IgA: 12 (7 II-A, 5 III-A); BJ: 7 (3 II-A, 4 III-A), BJ+IgG: 2 II-A, 1 III-A, BJ-IgA: 1 IIIA. Previous therapy: 1 schedule: 1 (15.4%), 2: 10(30.7%), 3: 17(50%), 4: 13(38%). For the analysis 36 patients were valuable. Response were reached in 76.9% (CR+PR), 65.4% (CR), 46.1% (PR), 19.2% (NR). Regarding IPI clin R 11.5%. Mean courses to reached response: 3.6. No relation to response and presence or not chromosomal aberrations were observed. At 24 months on follow-up 7 patients had dead (20.6%) and 11 (42.3%) maintained response without therapy. In 11 patients (42.3%) a combination of Bortezomib+Dexa or Melphalan were administrated by relapse or progression. Adverse events. Thrombocytopenia (grade 3: 5, grade 1: 46.1%, fatigue 5 (38.5%), peripheral neuropathy 4 (30.8%), constipation 3 (23%), diarrhea 2 (15.4%), ZHV 2 (15.4%), pneumonia 2 (15.4%), pyrexia 1 (7.6%), hypotension 2 (15.4%), grade 3 leucopenia 1 (7.8%). In 2 patients (15.4%) the therapy was disrupted by toxicity. Conclusions. Bortezomib in monotherapy induce a high rate of response (76.9%) in refractory MM. The response is achieved in the first 4 courses. It is recommendable to make combinations after the 4th course of Bortezomib if response does not achieved. No severe adverse effects have been observed with an incidence of reversible haematological side effects in 46.1% and mild non-haematological side effects in 52%.
MVD was significantly higher in MM pts in III CS than in the pts in I CS (67 ± 7.7 vs. 40 ± 6.0, p<0.001). Similar finding was observed in the comparison of pts with IPI 3 and IPI 1 (7 vs. 4.5, p<0.05). The expression of FGR-3 was found significantly higher in III CS than in I CS (47.5 ± 25.2%, p<0.05), and in pts with IPI 3 compared to the pts with I PI 1 (60 vs. 22.5%, p<0.001). Significantly strong expression of RANKL was detected in III CS and in pts with IPI 3, comparing to the pts with I PI 1 (95 vs 55%, p<0.05). This correlated with low expression of OPG in III CS (Me 26 vs. 43.5m, log rank, p<0.05). Similarly, the overall survival of pts with IPI 3 was significantly shorter compared to the pts with I PI 1 (19.5 vs. 36m, log rank, p<0.001). The assessment of the activity of angiogenesis, osteoclastogenesis and sensitivity to the growth cytokines represent important predictive factor with strong clinical relevance in terms of prognosis of myeloma and application of the various novel therapeutic strategies.

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EPIGENETIC MODIFICATIONS OF THE DLK1/GTL2 IMPRINTED GENES IN MULTIPLE MYELOMA AND WALDENSTRONDS MACROGLODULINEMIA; PRELIMINARY RESULTS

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Background. DLK1 and GTL2 are imprinted genes on human chromosome 14q22. DLK1 belongs to the Notch epidermal growth factor-like family of receptors and ligands, is paternally expressed and encodes a cell surface transmembrane protein, while GTL2 is maternally expressed and encodes a non-translated protein. Loss of imprinting (LOI) is an epigenetic error associated with tumorigenesis giving the neoplastic clone a growth advantage. Aims. The aim of the study was to investigate whether LOI of the differentially methylated region (DMR) of GTL2 promoter have a pathogenic role in multiple myeloma (MM) and Waldenström’s macroglobulinemia (WM). Methods. We have studied 9 newly diagnosed patients (7 MM - 2 WM), 5 male and 4 female, with median age 66.5 years (range 49-82). In the subset of the MM patients 1 (14.3%) presented plasma cell leukemia, 1 (14.3%) had indolent MM, 2 (28.6%) had stage II disease, and the resting 3 (42.8) had stage III disease. Cytogenetic analysis was performed only in 2 of the patients, and the karyotypes were apparently normal. DNA methylation pattern was determined by methylation-specific PCR of samples previously subject- ed to bisulphite-treatment, according to preestablished procedures. Subjects who have undergone bone marrow aspiration for diagnosis of thrombocytopenia, and after we had excluded hematological malignancies served as controls. Results. DNA methylation status of the deriving from both blood and bone marrow cells was evaluated. The normal pattern consists of 2 bands (alleles), namely one corresponding to the unmethylated paternal allele, (size 160 bp) and one corresponding to the unmethylated maternal allele (size 120bp). We found that alterations of the DMR were present in 5 (55.55%) of the patients, of which 1 (11.11%) had WM while 4 (44.44%) had MM. In the patient with the indolent MM we have detected the methylation abnormal pattern in both bone marrow and peripheral blood while in the remaining 4 patients the abnormal methylation pattern was detected only in periph- eral blood samples (absence of the unmethylated allele). No association was observed between disease stage and methylation status. Summary/conclusions. A total of 18 samples were studied and in 6 (55.55%) we have altered the methylation pattern. It is probable that LOI through epigenetic modifications in the DMR of the GTL2 gene represents a potential pathogenic mechanism in MM and WM. This is an ongoing study. We will study a larger number of patients in order to verify our preliminary findings. Moreover we will try to find if there is any correlation between disease stage and epigenetic alterations of these imprinted genes.

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A MULTICENTER RETROSPECTIVE ANALYSIS OF ADVERSE EVENTS IN KOREAN PATIENTS USING BORTEZOMIB FOR MULTIPLE MYELOMA

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1Gil Medical Center, Inchon, South-Korea; 2Seoul Nat. Univ. College of Medicine, Seoul, South-Korea; 3Catholic HSCT Center, Seoul, South-Korea; 4Kyoungdong National University Hospital, Daegu, South-Korea; 5Sungkyunkwan Unv. School of Medicine, Seoul, South-Korea; 6Kyunghee Univ. College of Medicine, Seoul, South-Korea; 7Inha University Hospital, Incheon, South-Korea; 8Kosin University Hospital, Busan, South-Korea; 9Pusan Nat. Univ. College of Medicine, Pusan, South-Korea; 10Soon Chun Hyang University Hospital, Seoul, South-Korea; 11Cyeong-Sang Nat. Univ. College of Med., Jinju, South-Korea; 12Ulsan University Hospital, Ulsan, South-Korea; 13Wonju College of Medicine, Wonju, South-Korea; 14Kangdong Sacred Heart Hospital, Seoul, South-Korea

Backgrounds. The proteasome inhibitor bortezomib has demonstrated clinical activity in patients with multiple myeloma (MM). Adverse events including thrombocytopenia and peripheral neurotoxicity affected 30% to 60% of patients overall, and interrupted therapy in 10% to 20%. There is no prior toxicity data available for Asian patients using bortezomib for MM. Aims. To evaluate the pattern of adverse events in patients treated with bortezomib for their MM. Methods. We reviewed the clinical records of patients with the diagnosis of MM from 25 centers in Korea using the NCI Common Toxicity Criteria version 3.0. The included patients were treated with bortezomib alone or in combination with other agents including thalidomide. Results. Ninety-five patients with MM were treated; patients had a median age of 60 years (range: 42-77). The median number of previous treatments was 3 (range: 0-10), 39% of patients had been treated with four or more major classes of agents including thalidomide (67%) and autologous stem cell transplantation (51%). Regimens included bortezomib only in 35 (40%), bortezomib plus dexamethasone in 34 (36%), and bortezomib plus a thalidomide-containing regimen in 23 (24%) patients. The analysis of patient response to therapy revealed: CR + nCR in 31 (38%) and PR in 30 (32%), for an ORR of 65% in 93 patients. The most common adverse events reported were thrombocytopenia (47%), sensory neuropathy (42%), anemia and leukopenia (both 31%). Thirteen patients (14%) stopped therapy due to adverse events; neurotoxicity in 8, infection in 4 and diarrhoea in 1 patient. A neuropathy, more than grade 2, was more frequent in patients who received 4 or more prior therapy regimens (17/87) compared to those receiving 3 or less (14/58). Also combination of thalido- mide was significantly correlated with neurotoxicity of grade 1–3 (p=0.001). We identified six therapy-related deaths (6%) within 20 days after the last dose of bortezomib. Causes of death were infection in 3, disease progression in 2 and suicide in 1 patient. Conclusions. The incidence of thrombocytopenia and neurotoxicity were similar, however gastrointestinal toxicities were relatively low in Korean patients compared to other regimens including thalidomide. Significant neuropathy was associated with the number of prior regimens and combination with thalidomide. These findings provide useful information for clini- cians and patients using bortezomib.

0769

FREQUENCY AND DISTRIBUTION OF TRISOMY 11 IN MULTIPLE MYELOMA PATIENTS: RELATION WITH OVEREXPRESSION OF CCND1 GENE AND T(11;14) TRANSLOCATION

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Backgrounds. CCND1 is an established oncogene located on chromosome band 11q13 for which genomic rearrangement or amplification leading to overexpression of the cyclin D1 protein is commonly found as a common lesion in many human cancer. In mantle cell lymphomas cyclin D1 protein is present in almost all cases and is activated by the characteristic t(11;14) translocation. Also 50% of human breast cancers exhibit cyclin D1 protein overexpression: in 20% of these tumours amplification of the 11q13 region is present but in the remaining cases overexpression cannot be explained by translocation. The copy number, suggesting that pathogenic activation of cyclin D1 can occur via additional mechanisms. In carcinomas (including colon, lung, oesophagus,
head and neck) and melanomas, cyclin D1 is activated by gene amplification and is associated with poor prognosis. CCND1 overexpression have also been found in 25-50% of multiple myeloma (MM) cases. A molecular classification of MM, named TC classification, stratifies patients into five groups (TC1-TC5) based on the presence of the recurrent IgH chromosomal translocations and cyclins D expression. Patients overexpressing CCND1 may be divided into two groups: TC1, characterized by the t(11;14) or t(6;14) translocation with overexpression of CCND1 or CCND3 and a non hyperdiploid status and TC2, with low to moderate levels of CCND1, absence of any primary IGH translocation and a hyperdiploid status. Aims. To assess CCND1 gene and cyclin D1 protein overexpression in a series of primary MM patients, to explore its relationship to the presence of the t(11;14), and to evaluate frequency and distribution of trisomy 11 in the different TC groups. Methods. Fluorescence in situ hybridization (FISH) analysis with specific probes for CCND1 gene amplification (probe mixture of cyclin D1 band 11q13 - CEP 11 bands 11p11-q11) and t(11;14)(q13;q32) were performed on CD138-purified plasma cells from bone marrows of thirty MM patients at diagnosis. Cyclin D1 protein expression and intensity was evaluate by immunohistochemistry. Results. FISH analysis revealed CCND1 overexpression in 14/30 cases (46.6%) and the presence of the t(11;14) translocation in 9/30 cases (30%) (Table 1). Patients with evidence of the t(11;14) showed strong nuclear staining for cyclin D1 (TC1 group) and 8 out 9 demonstrated CCND1 overexpression. The remaining 6 out 15 cases with increased CCND1 gene copy numbers lacked the t(11;14) and showed low to negative levels of cyclin D1 protein (TC2 group). Globally, the frequency of trisomy 11 was 40% (12/30 patients). It was demonstrated in 3 out 9 cases carrying the t(11;14) (TC1), 5 out 6 overexpressing CCND1 without the translocation (TC2) and 4 out 15 negative for both alterations (TC3-TC5). Conclusion. In our data, trisomy 11 don’t seems to cause directly overexpression of CCND1 as it is present in 4/15 patients without overexpression of CCND1 and in 3/9 patients carrying the t(11;14). One patient belonging to the TC2 group, overexpressing CCND1 without the translocation (TC2) and 4 out 15 negative for trisomy 11 also overexpressing CCND1. The frequency of trisomy 11 non overexpressing CCND1 can be divided into two groups: TC1, characterized by the t(11;14) or t(6;14) translocation with overexpression of CCND1 or CCND3 and a non hyperdiploid status and TC2, with low to moderate levels of CCND1, absence of any primary IGH translocation and a hyperdiploid status. Aims. 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One patient belonging to the TC2 group, overexpresses CCND1 and lacks both trisomy and translocation suggesting that cyclin D1 can be dysregulated by additional mechanisms. In TC2 group trisomy 11 probably may be considered as a recurrent polymorphism of the hyperdiploid status.

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*In the presence of CCND1 overexpression, the number of copies for each gene is indicated. NP: not performed; MC: monoclonal component. |H score >75% tumor cell positive; ++++ 50-75% tumor cells positive, 25-50% tumor cells positive, +10-25% tumor cells positive.
tation none of the patients had peripheral neuropathy > grade 1. Patients were scheduled to receive bortezomib 1.3 mg/m² IV (days 1, 4, 8 and 11) every three weeks for eight cycles and dexamethasone was planned to be added at a dose of 20 mg every other day (days 2, 5, 9, 12), if no signs of response were observed after the 1st cycle. Actually, 27 patients received dexamethasone and the 2 patients with plasma cell leukemia received thalidomide in addition. Results. Two patients in terminal resistant disease died prematurely and 3 patients are still receiving the 2nd cycle (thus, 5 patients are not evaluable at present). Overall, 39 out of 47 evaluable patients responded (83%) (3 complete remission [CR], 5 near CR, 25 partial response, 6 minimal response). The median time to response was 42 days. Within a median follow-up of 8 months (2-160), 16 (34%) patients relapsed and 8 patients (18%) died, 7 of disease and 1 of unrelated cause. In the majority of patients, non-neurologic toxicity was mild and reversible including fever, fatigue, gastrointestinal symptoms and hematologic toxicity. The most severe side effect was peripheral neuropathy which developed in 60% of patients. Neuropathy included ataxia, caustic pain and sensory disturbances and resolved after a median time of two months after discontinuation of Bortezomib. In most patients, treatment was reduced or stopped because of peripheral neuropathy but all responding patients completed at least 4 cycles. Conclusion. Bortezomib therapy alone or in combination with low dose dexamethasone produces rapid responses in relapsed and refractory MM. Early relapses are frequent. Neuropathy is the most important adverse reaction and lead to dose reduction or discontinuation of treatment.

0772
BORTEZOMIB AS A SINGLE AGENT IN REFRACTORY/RELAPSED MULTIPLE MYELOMA RESULTS OF CZECH MYELOMA GROUP (CMG)
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Summary. The efficacy of single agent bortezomib in the treatment of refractory multiple myeloma has been shown repeatedly. We summarize the results of bortezomib therapy in Czech republic. 82 patients with a median age 61 years (33-84) with refractory/relapsed myeloma were scheduled to receive Velcade 1.3 mg/m², on days 1, 4, 8 and 11 mostly of a 21-day cycle. This was a heavily pretreated population (1-8 prior therapies, median 2), including high-dose therapy with stem cells support (56 patients, 68%) and/or thalidomide (42 pts, 51%). The overall results, assigned by EBMT criteria, were as follows: the response was achieved in 40 patients (48.8%) - eight patients had complete (9%), 19 partial (29%) and 8 minor (9%) response. In 10 cases stabilization of disease was observed, 20 patients progressed during therapy, 5 died early after the start of therapy and in 7 cases the evaluation was not available due to short time of therapy. The response was observed early after the start of therapy in most cases, in 33 patients after the first cycle (40%) and in 11 after the second cycle (15%), although in minority of them progression during further therapy was observed. The most common adverse events were thrombocytopenia (65.9%) and neuropathy (52.4%), however, grade 3/4 thrombocytopenia developed in 37.8% and neuropathy only in 7.3% of patients, respectively. Other grade 3/4 complications were anemia (6%), granulocytopenia (13%), gastrointestinal events (8.5%), renal failure (6%) and infections (5%). Conclusion. Our experience confirmed that bortezomib provides clinical benefit with manageable toxicities in this heavily pretreated and high-risk population.
Chronic lymphocytic leukemia and related disorders
Clinical/Experimental II

**0774**
Dexamethasone induces apoptosis ex vivo in chronic lymphocytic leukemia cells with either unmutated IgVH genes or high ZAP-70 expression
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Hospital Clinic, Barcelona, Spain

Background. Patients with chronic lymphocytic leukemia (CLL) and unmutated IgVH genes or high ZAP-70 expression have poorer prognosis than those with mutated IgVH genes or low ZAP-70 levels. This is in part related to the resistance of unmutated and ZAP-70 positive cases to treatment agents that induce apoptosis in a p53-dependent manner. It has been suggested that corticosteroids are active in CLL through p53-independent pathways. Aims. To analyze the ex vivo response to dexamethasone in CLL cells according to the IgVH mutational status and ZAP-70 levels, and the expression of different glucocorticoid receptors in CLL cells. Methods. Frozen lymphocytes from 60 patients with CLL were analyzed for ZAP-70 expression and IgVH mutational status (n=44). Cells were cultured and treated using fludarabine (5 µg/mL), Mitoxantrone (0.5 µg/mL), FCM (fludara 1 µg/mç, maphosphamide 1 µg/mL and mito 0.5 µg/mL), FM (fludara 1 µg/mL and mito 0.5 µg/mL), Dexamethasone (5.2 µg/mL) and FMD (fludara 1 µg/mL, mito 0.5 µg/mL and dexa 5.2 µg/mL). Cell viability and apoptosis were determined by annexin/V/PI staining and FACS analysis at three different time points for each patient and conditions (0 h and 24 h without treatment, and 24 h with each treatment). The expression of glucocorticoid receptor (GR) isoforms α, β and γ was analyzed by Quantitative RT-PCR in 20 cases. Results. Dexamethasone-induced apoptosis was significantly higher in samples with unmutated IgVH genes and/or high ZAP-70 expression (≥ 20%) than in those with mutated IgVH genes and/or low ZAP-70 expression (median cell viability 65% vs. 81%, respectively, p<0.001). In contrast, the highest cell mortality induced by mitoxantrone was observed in samples with IgVH mutations or low ZAP-70 expression (p=0.009). Median cell viability was 56.1% for FM vs. 34.3% for FMD (p<0.0001) regardless of the IgVH mutational status. No differences in cell viability were found according to ZAP-70 expression or IgVH mutational status after ex vivo treatment with fludarabine or FCM (p=0.649 and p=0.055, respectively). No relationship was found among IgVH mutational status and the expression of the different GR isoforms. Expression of the three different GR isoforms was also similar in corticosteroids responders and non-responders. Conclusions. In this study, CLL cells with unmutated IgVH genes or high ZAP-70 expression showed a higher cell mortality after ex vivo exposure to dexamethasone than those with mutated IgVH genes or low ZAP-70 expression, with no relationship with the expression of the different GR isoforms. These data give conceptual support to trials aimed at determining the role of dexamethasone in the treatment of patients with CLL and poor prognostic features or resistant to fludarabine.
ZAP-70 EXPRESSION IN NEOPLASTIC CELLS AND T LYMPHOCYTES OF B-CLL PATIENTS: A REPRODUCIBLE METHOD FOR DETECTION USING FLOW CYTOMETRY

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Background. Prognostic stratification in B-CLL is critical to design informative therapeutic trials. The best combination of biological and clinical parameters for patient classification in prognostic groups should include ZAP-70 expression, which correlates with lack of somatic mutations of immunoglobulin VH genes. Determination of ZAP-70 expression by flow cytometry is attractive due to wide accessibility and speed, but is hampered by lack of standardization. Aim. The aim of this study was to identify the best strategy for flow cytometric analysis of ZAP-70 expression in B-CLL cells, and to compare it with other prognostic factors. Methods. ZAP-70 expression was determined in PB samples of 59 patients (19 males, 20 females; median age 65.3, 51-85 years), including 23 stage A and 16 stage B or C. ZAP-70 expression was determined within 24 h of sample drawing. In 18 patients the determination was performed before treatment. Anti-CD19, CD20, CD22, CD23, FMC7, CD79b, CD38, CD3, CD5, Zap-70 (PE-labeled), K and L fluorescent monoclonal antibodies were used, and a minimum of 5000 events were acquired. We determined the percentage of B-CLL cells expressing ZAP-70 with intensity equal or higher than T cells. If Zap-70 was expressed in more than 20%, tumor cells were considered positive. To reduce inter-observer and technical variability, we further calculated the ratio between the median fluorescent intensity (MFI) for ZAP-70 in B-CLL and T cells. The relationship with CD38 expression (positive if present in more than 30% of neoplastic cells) and genetic abnormalities (deletion of 13q, 11q, 17p, and trisomy 12, evaluated by FISH) was determined using the chi test. Time-to-first-treatment was calculated using the Kaplan-Meier method and the influence of ZAP-70 expression evaluated by the log-rank test. Results. With a median follow-up of 54.7 (0-145) months only one patient died. Median time-to-first-treatment was 11.3 (0-132.9) months, with 7 patients receiving fludarabine-based regimens, 12 alkylating agents, and 2 anticyclines (global response rate 62%). Patients were divided in 3 groups according to the presence of the following cytogenetic findings: del17p and/or del11q (20.5%), trisomy 12 or no abnormalities (38.5%), and del13q (23.1%). Seventy-four percent were ZAP-70 positive, while only 36% were CD38 positive. ZAP-70 positivity was unrelated to Binet stage, CD38 expression and cytogenetic findings (p=0.335). Time-to-first-treatment was similar in ZAP-70 positive and negative patients (p=0.99). However, when the ratio between MFI of ZAP-70 in B-CLL and T cells was used, we found that patients with more than higher than 0.4 had a prolonged time-to-first-treatment as compared to patients with lower ratios (p=0.03). Conclusions. Determination of ZAP-70 expression using the ratio between ZAP-70 MFI in tumor and T cells is a reproducible method for ZAP-70 evaluation in B-CLL, by simultaneously providing an internal control for the fluorescence intensity of positive cells and reducing the inter-observer and technical variability.

WNT5B EXPRESSION: A NEW POTENTIAL PROGNOSTIC MARKER IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. ZAP70 and CD38 expression, along with heavy chain (IgH) mutational status, are currently under investigation as predictive markers in chronic lymphocytic leukemia (CLL). Previous data (Deseng et al PNAS 2004) suggested that Wnt signaling pathway contributes to a deficient apoptosis in CLL. Wnt5b is a ligand for members of the frizzled family of seven transmembrane receptors. It may be a signaling molecule which affects the development of some regions of tissues. Correlation of ZAP70, CD38 expression and IgH status with Wnt5b expression. Method. This retrospective study was conducted on 14 newly diagnosed and 9 relapsed CLL patients and on 18 healthy donors. We used the ABI PRISM 7000 Real Time platform to analyze Wnt5b expression. Results. All patients showed Wnt5b expression, while 8 CLL patients and 1 healthy donor were negative. We also evaluated CD38 (Ibrahim et al Blood 2001). ZAP70 expression (Crespo et al N Engl J Med 2003) and IgH rearrangement (Theriault et al Mod Pathol 2000) as previously described. Results. All healthy donors and 15 CLL patients showed Wnt5b expression, while 8 CLL patients were negative. After a median follow-up of 21.5 months (range 5-29), 6 patients were in complete remission, 11 were in stable disease (SD) and 6 had progressive disease (PD). 14/15 patients (96%) with Wnt5b expression at diagnosis were in PD or SD (p=0.01 vs patients in CR) at the end of follow-up. All patients who achieved a CR after therapy had no or very low Wnt5b expression at diagnosis (medi-
This study was designed to determine the frequency of CMV. Molica, S. Matis, M. Spriano, and M. Gentile, D. Cutrona, G. Festini, C. Gentile, M. Ferrarini, E. Rossi, and H.D. Alexander, C. Matthews, M.A. Catherwood, T.C.M. Morris, Belfast City Hospital Trust, Belfast, Northern Ireland.

The background of chronic lymphocytic leukaemia (CLL) is characterised by a profound dysregulation of the host’s immune system. This results in recurrent infections and an increased incidence of autoimmune diseases, suggesting T-cell dysfunction. Furthermore, the frequent use of potentially more immunosuppressive therapies such as fludarabine and alemtuzumab, has also increased the incidence of recurrent infections, such as cytomegalovirus (CMV). In CLL, CMV is a member of the herpes family of DNA viruses and arguably causes the most morbidity and mortality of any herpes virus. CMV establishes life-long latent infection without clinical disease in immunocompetent individuals. For those with the virus, the disease may manifest as a second malignancy, with a significant risk of disease progression and death. CMV infection may be involved indirectly in sustaining the tumour. Finally, the intriguing finding of high CMV DNA copy numbers in patients with VH3-21 rearrangements suggests that CMV infection may play a role in the poor outcomes in these patients.

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**T CELL SUBSETS AND CMV INFECTION IN CLL: ASSOCIATIONS WITH IGH MUTATIONAL STATUS, GENE USAGE AND CHROMOSOMAL ABERRATIONS**

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**Background.** Chronic lymphocytic leukaemia (CLL) is characterised by a profound dysregulation of the host’s immune system. This results in recurrent infections and an increased incidence of autoimmune diseases, suggesting T-cell dysfunction. Furthermore, the frequent use of potentially more immunosuppressive therapies such as fludarabine and alemtuzumab, has also increased the incidence of recurrent infections, such as cytomegalovirus (CMV). In CLL, CMV is a member of the herpes family of DNA viruses and arguably causes the most morbidity and mortality of any herpes virus. CMV establishes life-long latent infection without clinical disease in immunocompetent individuals. For those with the virus, the disease may manifest as a second malignancy, with a significant risk of disease progression and death. CMV infection may be involved indirectly in sustaining the tumour. Finally, the intriguing finding of high CMV DNA copy numbers in patients with VH3-21 rearrangements suggests that CMV infection may play a role in the poor outcomes in these patients.

**Aims.** This study was designed to determine the frequency of CMV DNAemia in CLL patients, to assess the association between CMV DNAemia and T-lymphocyte subset numbers, and to investigate associations between CMV DNAemia and other parameters associated with poor prognosis in CLL.

**Materials and Methods.** This was a retrospective analysis of 91 patients from the Belfast CLL study cohort, which consisted of 71 newly diagnosed Binet stage A patients, 31 previously diagnosed stage early stage patients and 15 cases that had advanced CLL (Binet C/Rai III/IV). Significantly higher serum CMV levels were found in IgVH unmutated, compared to IgVH mutated, patients (p<0.001). Elevated serum CMV levels were also associated with CD38 positivity (p<0.013) and poor and intermediate prognosis chromosomal abnormalities (p<0.001). A TK level of greater than 8.5 U/L, best identified patients with progressive disease. Within the newly diagnosed group, nine IgVH mutated cases had a TK level of 8.5 U/L, or greater. Closer scrutiny revealed that these patients had either VH3-21 or VH-69 gene usage and/or had short LDT of <12 mths, which is associated with poorer outcomes. Additionally, within the unmutated subgroup of newly diagnosed patients, only one had a TK level lower than 8.5 U/L. This particular patient had not undergone lymphocyte doubling, greater than four years after diagnosis, which was longer than that seen in the remaining unmutated cases, with the highest TK values recorded in patients with lymphocyte doubling times. **Conclusions.** Unlike IgVH mutational status, TK does not lose predictive power as disease progresses, with the highest TK levels reported in advanced clinical stage. This study demonstrated that determining serum TK level at diagnosis in early stage patients can identify those most likely to progress and therefore require earlier treatment. Furthermore, the variations in disease progression within prognostic subcategories can be predicted by measuring serum TK levels at diagnosis, allowing further refinement of risk stratification. We confirm the efficacy of TK measurement in CLL to determine proliferation activity and predict clinical course of this heterogeneous disease.

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**DIAGNOSTIC POTENTIAL OF CD38 COMBINED WITH ZAP-70 EXPRESSION IN PREDICTING MUTATIONAL STATUS OF IMMUNOGLOBULIN HEAVY-CHAIN VARIABLE REGION IN 450 CHRONIC LYMPHOCYTIC LEUKAEMIA CASES**


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**Backgrounds.** Recent advances in the diagnosis and molecular characterisation of CLL permit improved prediction of disease prognosis, which could result in better management. The best-studied parameters are somatic hypermutation of the immunoglobulin heavy-chain variable region (VH), expression of the cellular proteins CD38 and ζ-association. CD38 expression is a hallmark of progressive disease. This study investigated associations between CD38 expression and ζ-association in newly diagnosed Binet stage A patients. Furthermore, the use of serum TK measurement to identify subcategories of disease within those defined by IgVH mutational status, gene usage, CD38 expression, Zap-70 positivity and chromosomal abnormalities, was studied. **Methods.** Ninety-one CLL patients were recruited to this study. Serum TK levels were measured using a radioenzyme assay. IgVH mutational status and VH gene usage were determined using BIOMED-2 primers and protocol. Recurring chromosomal abnormalities were detected by interphase fluorescent in-situ hybridisation (FISH). CD38 and Zap-70 expression were determined by flow cytometry and RT-PCR respectively. Results: Nineteen of the 91 patients were newly diagnosed Binet stage A patients, 31 were previously diagnosed early stage patients and 15 cases that had advanced CLL (Binet C/Rai III/IV). Significantly higher serum CD38 levels were found in IgVH unmutated, compared to IgVH mutated, patients (p<0.001). Elevated serum CD38 levels were also associated with short LDT of <12 mths, which is associated with poorer outcomes. Additionally, within the unmutated subgroup of newly diagnosed patients, only one had a TK level lower than 8.5 U/L. This particular patient had not undergone lymphocyte doubling, greater than four years after diagnosis, which was longer than that seen in the remaining unmutated cases, with the highest TK values recorded in patients with lymphocyte doubling times. **Conclusions.** Unlike IgVH mutational status, TK does not lose predictive power as disease progresses, with the highest TK levels reported in advanced clinical stage. This study demonstrated that determining serum TK level at diagnosis in early stage patients can identify those most likely to progress and therefore require earlier treatment. Furthermore, the variations in disease progression within prognostic subcategories can be predicted by measuring serum TK levels at diagnosis, allowing further refinement of risk stratification. We confirm the efficacy of TK measurement in CLL to determine proliferation activity and predict clinical course of this heterogeneous disease.

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**SERUM THYMIDINE KINASE LEVELS CAN IDENTIFY EARLY STAGE B-CLL PATIENTS WITH MUTATED IGVH GENES MOST LIKELY TO PROGRESS**

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**Backgrounds.** Thymidine kinase (TK) is a cellular enzyme which functions as part of the pyrimidine salvage pathway involved in DNA synthesis. Serum TK levels have been shown to be correlated with survival in many malignancies, including CLL. Aims. This study was designed to investigate associations between TK levels and other prognostic markers, in newly diagnosed Binet stage A patients. Furthermore, the use of TK measurement to identify subcategories of disease within those defined by IgVH mutational status, gene usage, CD38 expression, Zap-70 positivity and chromosomal abnormalities, was studied. **Methods.** Ninety-one CLL patients were recruited to this study. Serum TK levels were measured using a radioenzyme assay. IgVH mutational status and VH gene usage were determined using BIOMED-2 primers and protocol. Recurring chromosomal abnormalities were detected by interphase fluorescent in-situ hybridisation (FISH). CD38 and Zap-70 expression were determined by flow cytometry and RT-PCR respectively. Results: Nineteen of the 91 patients were newly diagnosed Binet stage A patients, 31 were previously diagnosed early stage patients and 15 cases that had advanced CLL (Binet C/Rai III/IV). Significantly higher serum TK levels were found in IgVH unmutated, compared to IgVH mutated, patients (p<0.001). Elevated serum TK levels were also associated with short LDT of <12 mths, which is associated with poorer outcomes. Additionally, within the unmutated subgroup of newly diagnosed patients, only one had a TK level lower than 8.5 U/L. This particular patient had not undergone lymphocyte doubling, greater than four years after diagnosis, which was longer than that seen in the remaining unmutated cases, with the highest TK values recorded in patients with lymphocyte doubling times. **Conclusions.** Unlike IgVH mutational status, TK does not lose predictive power as disease progresses, with the highest TK levels reported in advanced clinical stage. This study demonstrated that determining serum TK level at diagnosis in early stage patients can identify those most likely to progress and therefore require earlier treatment. Furthermore, the variations in disease progression within prognostic subcategories can be predicted by measuring serum TK levels at diagnosis, allowing further refinement of risk stratification. We confirm the efficacy of TK measurement in CLL to determine proliferation activity and predict clinical course of this heterogeneous disease.
characteristic ROC curves, which were generated by calculating the sensitivity and specificity of each cut-off point. Moreover, the usefulness of CD38 and ZAP expression in identifying VH mutational status was assessed using the following standard diagnostic tests: sensitivity and specificity, positive and negative predictive values and accuracy, as well as by Kappa statistic. Results. As a first step, we determined, by ROC curve analysis, 30% as the best cut-off value which discriminated between mutated and unmutated cases in CLLs (area under the curve 0.758, p<0.0001). On the basis of standard diagnostic tests, CD38 expression, categorized by 30% cut-off value, had relatively low sensitivity (70%), specificity (77%), negative predictive value (76%) and positive predictive (71%) values in anticipating VH mutational status. Moreover, Kappa statistic revealed that the agreement between CD38 expression and VH mutational status was low although significant (K=0.47, p<0.001). On the other hand, ZAP-70 showed very low sensitivity (57%), high specificity (89%), low positive predictive value (57%), relatively low negative predictive value (72%) and a low, although significant, K statistic (0.47, p<0.001). Furthermore, we combined the value of both tests to evaluate whether both variables provided more precise information in estimating VH mutational status compared to that obtained from single test. In this regard, we obtained the following Results. sensitivity, 90%; specificity, 96%; positive predictive value, 90%; negative predictive value, 76%; K statistic 0.45, p<0.001. Moreover, ROC analysis was also performed to detect the optimal threshold IgV gene mutation capable of predicting lesions with positivity of both CD38 and ZAP-70. The best cut-off value was 1.9% (AUC 0.814, p<0.0001), which is close to the threshold (2%) used to distinguish mutated from unmutated B-CLL. Conclusion/Summary. Our data demonstrated that neither CD38 nor ZAP-70 by themselves had an important impact in anticipating VH mutational status. When CD38 and ZAP-70 were combined, the specificity and sensitivity improved, meaning that the combined use of CD38 and ZAP-70 could surrogate the prognostic value of VH mutational status. This information should be validated on clinical ground.

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VH3-48 AND VH3-53 GENE REARRANGEMENTS REPRESENT UNIQUE SUBGROUPS IN CLL AND ARE ASSOCIATED WITH BIASED A LIGHT CHAIN RESTRICTION, HOMOGENEOUS LCDR3 SEQUENCES AND POOR PROGNOSIS

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Backgrounds. In recent years IgVH mutational status, VH gene usage, and the potential role of antigens in the leukemogenesis of chronic lymphocytic leukemia (CLL), have been studied extensively. In particular, the identification of VH3-21 gene rearrangement, a unique subset of B-CLL patients, has lead to questioning of the prognostic limitations of IgVH mutational status, as VH3-21 usage is associated with poor prognosis, irrespective of the fact that two thirds of such patients have mutated IgVH genes. Furthermore, this specific gene has been linked with highly homogeneous heavy and light complementarity determining regions (CDR5), indicating that these patients possess virtually identical BCR binding sites and thus suggesting a common antigenic progenitor. Aims. The aims of this study were to scrutinize VH gene usage in a large cohort of CLL patients to determine if other VH gene rearrangements could identify similar prognostically significant subgroups of patients. Furthermore, we sought to identify the frequency of VH3-21 rearrangements in a Northern Irish population of CLL patients, and determine if chromosomal aberrations associated with poor outcomes could account for the poor prognosis in these patients. Methods. Two hundred and twenty eight patients were recruited from Belfast City Hospital Haematology Outpatient Clinic and surrounding regional hospitals. Clinical staging (Rai and Binet), immunophenotyping, lymphocyte doubling time (LDT) and time to treatment (TTT) were available on all patients. IgVH and IgVL mutational status, gene usage and CDR3 sequences were determined using multiplex BIOMED-2 primers and protocol and sequence analysis. FISH analysis was performed on all patients. Results. VH3-21 gene usage (n=18) was associated with poor prognosis, overuse of VL3-21 (VH3-14) gene and highly homogenous heavy and light CDR3s sequences. Only one VH3-21 patient showed an adverse prognosis chromosomal aberration. VH3-48 (n=8) and VH3-53 (n=4) gene rearrangements showed biased lambda light chain restriction (7/1, 1/3, 1/1, respectively). Further analysis revealed overuse of VL3-21 (VX2-14) gene (7/8) and highly homogenous LCDR3 sequence (QVDWSGSDHPW) in VH3-48 patients. Both VH5-46 and VH5-53 categories have a preponderance of females, short LDT (<21 months) and an absence of any poor prognosis chromosomal aberrations. Conclusions. This study shows that the incidence of VH3-21 usage in Northern Ireland (7.9%) lies between that reported in Scandinavian (12%) and Mediterranean populations (0-3%). Furthermore, VH3-48 rearrangements, together with VH3-21 and possibly VH3-53, represent unique subgroups in CLL associated with poor prognosis, irrespective of mutational status. The recurrent use of specific VL genes and homogenous LCDR3 sequences in these patients suggests a common aetiological factor.

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IDENTIFICATION OF NEW GENOMIC ALTERATIONS IN CLL USING A 32K BAC CGH ARRAY

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Backgrounds. Cytogenetics in CLL is hampered by low mitotic index. Genetic screening for prognostic factors has been routinely performed by FISH for established abnormalities. All of them are unbalanced, and some (e.g., del13q14) are believed to be early events of pathogenesis, whereas others (del17p) may be associated with progression of disease. To get a more detailed profile of genomic alterations in CLL we applied a genome wide arrayCGH. Aim: To screen the genome of CLL cells for novel genomic copy number alterations with high resolution 32 K BAC array. Method: We chose a genome wide array CGH BAC array produced by Swegene DNA Microarray Resource Center, Department of Oncology, Lund University, Sweden (http://swegene.onk.lu.se). The array covered the human genome with a tiling resolution of ~100 Kbp. The array data was analyzed with BioArray Software Environment (http://bioarray.bio.lub.lu.se). Results. Preliminary analysis of the DNA copy number profiles allowed detection of previously described genomic changes along with identification of novel alterations. The most common alteration among the CLL samples was del13q14 (54%) followed by del11q22 (30%). In 15 out of 28 samples displaying the del13q14 homozygous deletion was implied. The minimal deleted region (MDR) could be mapped to a region of 81 Mb encoding the genes DLEU1, DLEU2 and DLEU7. Losses of chromosome 11q spanned from 11q14.1 to 11q23.2 with the peak at 11q22.3. Trisomy 12 was detected in 25% of the samples, in several samples indicating only partial gains. Loss of the 17p arm was also detected, in some cases with a concurrent gain of the 17q arm. Genomic changes were detected in the majority of samples. For example, losses on 2q36, 5q13.2-12, 18q21.2 were detected in individual samples. Recurrent gains were mapped to 6q21.3 and 8q21.2. Summary/Conclusions. The high resolution CGH array combines full genome screening with high specificity and allows detection of small lesions. Genomic abnormalities were identified in most patients, including novel not yet well defined changes. The presented analysis is the first step toward the identification of novel changes and correlation studies will follow, and may improve our understanding of genomic lesions and their clinical implications in CLL.

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EVALUATION OF TRANSFERRIN RECEPTOR 1 AND 2 EXPRESSION AT THE RNA AND PROTEIN LEVEL IN NORMAL B CELLS VS. CHRONIC LYMPHOCYTIC LEUKEMIA

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Transferrin receptor 1 (TR1, CD71) is one of the classical activation markers up-regulated upon B-cell activation. TR2 has significant shared gene usage with TR1 and also binds transferrin, albeit with lower affinity than TR1. The TR2 gene has two alternatively spliced transcripts (α and β); in normal tissues, TR2-α mRNA is more abundant than TR2-β mRNA. Chronic lymphocytic leukemia (CLL) is characterized by almost ubiquitous CD71 expression (Smilvekska et al., Leuk Res. 2006;30:183-9), this is in keeping with the activated status of CLL cells. In the present study, we evaluated TR1 and TR2 expression at the mRNA and protein level in 76 CLL patients as well as CD19+ B cells.
TOSO were over expressed in CLL cases (CLL x nCD19 p=0.0130; CLL x MCL p=0.0357). The expression of TOSO correled significantly with BCL2 and ZAP70 expression in CLL (TOSO x BCL2, Spearman’s r=0.5439, p=0.0019; TOSO x ZAP70, r=0.5318 p=0.0025). CFLAR was over-expressed both in CLL and MCL when compared with nCD19+ cells (p=0.01). Conclusions. This new approach to evaluate gene expres- sion revealed that both CLL subtypes have different expression profiles. Various differences of the gene expression observed between the CLL and its nor- mal counterpart are related to the deficiency of apoptosis, and among them we demonstrate for the first time the participation of TOSO and CFLAR. They represent additional pathways that contribute to apoptosis inhibition, and the expression of TOSO is probably associated with a poor outcome. Our findings strengthen the role of aberrations in the pathways regulating in the genesis of the leukemic phenotype, and reveal novel genes involved in these pathways.

T-CELL TYPE LYMPHOPROLIFERATIVE DISEASE OF GRANULAR LYMPHOCYTES IS EQUIPPED WITH A PHENOTYPIC PATTERN TYPICAL OF EFFECTOR CYTOTOXIC CELLS

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In 22 patients with CD3- DLGL we investigated whether proliferat- ing GL displayed the phenotype of fully differentiated cytotoxic cells. To this aim, according to the recently defined phenotypic pattern recog- nizing the different stages of cytotoxic effector cell differentiation, we also evaluated, by real-time PCR, the expression of BCL2, ZAP70, CD27, CD28, CD45RA, CD69, CD62L, CCR7, IFN-γ on GL surface. Similar NK receptors have been found to be central in the pathogenesis of DLGL, we also analyzed the expression of Killer-Immunoglobulin-like Receptors (KIRs), CD94/NKG2, NKG2D and Natural Cytotoxicity Receptors (NCRs) on GLs surface. In all DLGL patients taken into account, GLs were found to be mononotypically reared for the T-cell receptor (TCR). In 18/22 patients studied, our data showed that CD3-CD16+ cells expressed a homogenous CD45RA+; CD27+, CD28+, CD62L+, CCR7, INF-γ pattern, consistent with those of fully differentiated CTLs. In four cases a coexpression of CD45RA and CD45RO was documented. The majority of these patients (20/22) expressed NKG2D receptor. In addition, KIR receptors were expressed only in a fraction of patients (7/22) as far as CD94/NKG2 was (5/22). Interestingly, the activating receptor CD94/NKG2 was detected in 2/5 of CD94- cases, suggesting that activating signals for cell proliferation might stem from this receptor. In all patients’ GLs the NCRs NKP44, NKP46, NKP80 were absent, while NKP80 was expressed in the majority of cases (20/22). In conclusion, our data demonstrated that GLs in CD3- DLGL patients show a phenotype consistent with that of fully differentiated CTL. The expression of NK receptors, although useful for the definition of diagnosis of DLGL, does not represent a critical feature of the abnor- mal clone, suggesting that expression of these receptors is independent from the acquisition of the in vivo mature CTL phenotype, which indeed represents the truly distinctive phenotypic hallmark in these patients.

LOW-DOSE ALENTEZUMAB MONOTHERAPY IN ADVANCED CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Backgrounds. Alentuzumab is a well established therapeutic tool in CLL and its low-dose (ld) subcutaneous (sc) administration is safe and efficacious. Aim: To confirm on a larger number of patients with a longer follow-up the results of an already published (Cortezi, A. et al. Haema- tologica 2005, 90: 410-412) pilot study on ld sc alemtuzumab in retrac- tion of resistance. Methods. Since June 2004, we instututed the thirty-six consecutive CLL patients, 33 of whom are evaluable for efficacy. Patient characteristics were as follows: M/F 22/14; median age 68 years, range 48-83; Binet stage C 36.1%, stage B 56.6%, progressive stage A 8.3%; abnormal karyotype 69.4%, including unfavourable alterations (17p- 13.8%, 11q-22.5%, trisomy 12 19.4% and 6q-5.5%); ZAP70 positive 28/30 (93.3%). All the patients were pretreated (median prior lines of therapy 2, range 1-5) and refractory to alkylators, 58.3% were fludarabine-resistant, and 25% also rituximab-resistant. Previous grade 3/4 infections were documented in one third of the patients.

NEW PATHWAYS INVOLVED IN APOPTOSIS INHIBITION IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Apoptosis reduction mediated mainly by increased expression of BCL2 is a major mechanism of accumulation of mature B lymphocytes in peripheral blood (PB) and bone marrow in chronic lymphocytic leukemia (CLL). The disease comprises two subtypes that are characterized by important outcome differences observed between patients expressing either mutated (MUT) or unmutated (NAIVE) immunoglobulin genes. Aim: We analyzed the gene expression of the malignant cells of CLL (MUT and NAIVE) to identify possible additional- molecular mechanisms related to decreased apoptosis. Materials and methods. CD19+ lymphocytes were isolated by positive magnetic cell sorting (MACS) from six CLL patients (3 MUT and 3 NAIVE), three mantle cell lymphoma (MCL) patients, and 4 normal naive B-lympho- cytes isolated from tonsils. Gene expression profiles were obtained by serial analysis of gene expression (SAGE) and individual gene expression were measured by real time PCR and semi-quantitative RT-PCR. We also evaluated, by real-time PCR, the expression of BCL2, ZAP70, CFLAR, CERDOSO, FAS (FAIM5) in 22 CLL, 8 MCL patients and 8 normal CD19+ PB cells (nCD19+) obtained from healthy volunteers. Results. Approximately 100,000 tags were sequenced for each CLL subtype, corresponding to 15,000 known genes, whereas for MCL and normal naive B cells we sequenced about 60,000 tags which matched 16,000- 15,000 known genes. The comparison between the two subtypes of CLL revealed only 27 genes with significant (p<0.001) differences. The comparison of the transcriptional profiles from CLL with that of nCD19+ (obtained from Hashimoto et al. 2003) revealed that transcripts of IL4R, IL24, TOSO and FMO3 were exclusively or highly represented in CLL, whereas Jun, Jun-B, IL-8 and EGR-1, among others, were poorly rep- resented and was up-regulated in nCD19+. Comparison of MCL and naive B lymphocytes revealed that CFLAR was overrepresented in MCL (p<0.001). To corroborate SAGE results, observed differences in gene expression levels were vali- dated by semi-quantitative RT-PCR of the same six cases of CLL, 3 cases of MCL, one nCD19+ cells and one normal bulk BM, for 7 different genes: HLA-DR, 5, SIAT-5, SURF-5, IL4R, FMO4, IL24 and IL8. The real- time PCR analysis of 22 CLL, 8 MCL patients and 8 nCD19+ reveals that
Alectuzumab schedule was as follows: 10 mg sc X 3/week for 18 weeks, with anti-infective prophylaxis. Results. The overall response rate (NCIWG criteria) was 48.5%, including 21.2% complete response. Better responses were observed in blood (77.4%), as compared with bone marrow (54.5%), spleen (50%), and lymph nodes (48.5%). Responses were observed in patients with Zap7o+ (45%), adverse Karyotype (40%), 17p- (60%), stage C (46%), fludarabine- (47.4%) and rituximab-resistant disease. Responses on was achieved in 7/16 patients after a median of 12 months (range 6-18 months). After a median follow-up of 15 months (range 1.5-38) the median survival has not been reached for the entire case series (66.6% alive), responders (75%), and non-responders (58.8%). Eleven patients died after a median of 10 months (range 1.5-25) for infectious complications. 2 of them were in remission and 9 had active disease. Grade III-IV neutropenia or anemia were recorded in 36.1% and 2.7% of the patients, respectively. Mild anaemia was observed in 55.5% of the patients during alectuzumab therapy. Coombs-positive AIHA was documented in two patients after the end of Tx administration (9 and 1.5 months), being a reactivation in one of them. Two patients developed ITP one month after stopping alectuzumab. Infections (10 isitis media due to Pseudomonas, 1 dermatomieric Herpes zoster, 2 pneumonia, 1 lethal polymicrobial sepsis) occurred during alectuzumab treatment in 13.8% of the patients. Transient and clinically silent reactivation of cytomegalovirus was documented in 22.2% of the patients by pp65 antigenemia testing or PCR. Eight patients (4 with and 4 without reactivated HBV infection) received lamivudine while on alectuzumab. Adefovir dipivoxil was associated to lamivudine in two cases for an hepatitis flare. These antiviral therapies enables us to complete alectuzumab Tx in all the patients. Conclusions. We confirm on a larger number of high-risk relapsing/refractory CLL patients the high percentage of response, long remission duration, and the favourable toxicity profile of id sc Alectuzumab already shown in the pilot study.

**0789**

**ALLOGENEIC STEM CELL TRANSPLANTATION AND CHRONIC LYMPHOCYTIC LEUKAEMIA: DISTINCT IMMUNoglobulin VARIABLE HEAVY CHAIN GENES AS A POSSIBLE PROGNOSTIC INDICATOR**

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**Backgrounds.** Although the treatment of chronic lymphocytic leukaemia (CLL) has changed dramatically over the previous few years, CLL remains an incurable disease. Great proportions of patients relapse early after the treatment and eventually become refractory to treatment. To a number of these subjects allogeneic stem cell transplantation (alloSCT) is being considered as the alternative treatment option. Significant data in the literature has shown that CLL patients who are in complete remission have a higher probability to achieve long-term remission after SCT. However, the data are still considered as an early experimental approach and is associated with a number of risks. The mutational status of the immunoglobulin variable heavy chain (VH) genes is a major prognostic indicator for the clinical course and outcome of the CLL patients. More recently, additional prognostic categories have been identified by recognizing disease subsets which utilize unique VH genes. Examining the matched sets of VDJ, VDJ-32, VDJ-42, VDJ-54, VDJ-62 and VDJ-72 genes which are invariably associated with progressive and stable disease, respectively. Aims. The aim of our study was to investigate the possibility to identify suitable CLL candidates for alloSCT among CLL subsets that utilize unique VH genes. Methods. The study group consisted of 106 consecutive CLLs that had been diagnosed at our Institution prior to 2000, according to standard morphologic and immunophenotypic criteria. VH gene family usage and mutational status were obtained by direct sequencing of RT-PCR amplified RNA samples. Correlations between the different CLL subsets were made using standard statistical tests. Results. Our results showed that 61 (57.6%) patients utilize mutated VH genes, and the rest 45 (42.4%) have unmutated sequence. The most frequently rearranged VH gene in our CLLs was V1-69 gene, in all 25 cases (23.6%) with unmutated sequence. We compared the overall survival (OS) of the V1-69 subgroup against all the other patients. The two groups were comparable regarding the sex, age, total tumor mass (TTM) score and Rai stage. The VH-69 group has median OS of 56.7 months and all others patients have median OS of 125.8, (p=0.0001). Then, we further analyzed the differences in survival between VH1-69 cases and all patients with unmutated VH genes. There were no differences between the two subgroups regarding the age, gender, TTM score, Rai stage and OS. No other unique VH gene, utilized in our study group, had frequency important of analyzing. Conclusion: Our data do not support the thesis that patients expressing V1-69 gene form a distinct subgroup of CLL patients. Further investigations are needed to reach the definitive conclusion regarding the role of V1-69 gene and all others distinct VH genes in the prognosis and treatment decision in CLL patients. Our results confirmed that CLL with unmutated VH gene sequence has poorer OS and we suggest that all younger patients that utilize unmutated VH genes should be consider as candidates for early alloSCT, immediately after the first complete remission, if HLA identical donor is available.

**0790**

**REVERSAL BONE MARROW ANGIOGENESIS AFTER CONSOLIDATION THERAPY WITH ALECTUZUMAB IN ADVANCED CLL**

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We have previously shown that in patients with advanced chronic lymphocytic leukaemia (CLL) who respond to therapy with fludarabine bone marrow (BM) angiogenesis decreases significantly. To expand on these observations, we evaluated microvessel area of BM samples from 20 patients with advanced CLL (i.e., symptomatic Binet stage B or C) who received at least 8 weeks after the end of treatment with fludarabine subcutaneous alectuzumab, three times weekly for 6 weeks, at escalating dose up to 10 mg. The patient sample included 14 males and 6 females with a median age of 51 years (range 44-60). After a median number of 6 cycles of fludarabine (range, 4-15), 11 (55%) patients could be classified in complete remission (CR) and 9 (45%) in partial remission (PR) (7 nodular-PR and 2 PR). Interestingly, the rate of CR increased to 90% (18 CR; P=0.03, Fisher’s exact test) after treatment with alectuzumab. In keeping with hematological responses, significant changes of BM angiogenesis were observed. The assessment of microvessel area carried out at the starting of therapy, after fludarabine and at the end of therapy with alectuzumab, respectively, showed a continuous decrease in the extent of microvessel area (p=0.02). This conspicuous feature was easily demonstrable in ZAP-70-positive (p=0.02) and ZAP-70-negative (p=0.0001) patients. As far as molecular response is concerned, 13 out of 20 (65%) patients changed from a monoclonal to a polyclonal pattern of IgH. A separation evaluation carried out in patients with a persistent monoclonal IgH pattern and in patients who changed to polyclonal pattern of IgH after therapy with alectuzumab showed a significant reduction of BM microvessel area only in the latter (p=0.0002). Finally, a significant (p=0.0001) decrease of the extent of BM angiogenesis was observed among patients who received a cumulative dose of alectuzumab higher than median (i.e., symptomatic Binet stage B or C) while the same did not apply for those who had received cumulative dose of alectuzumab lower than median (p=0.127). In conclusion, a decrease in BM vascularity was observed after treatment with alectuzumab. Such a finding reflects either molecular response or cumulative dose of alectuzumab. These observations lend support to the anti-angiogenic role played by alectuzumab in CLL.

**0791**

**FLUDARABINE AND CYCLOPHOSPHAMID (FC) VERSUS CYCLOPHOSPHAMID, VINCristine AND PREDNISONE (CVP) AS FIRST LINE TREATMENT IN CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL)**

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**Backgrounds.** The combination of CVP is known to be active in previously untreated CLL patients. FC is another effective regimen. Aim: To evaluate the response rate, time to disease progression and survival of FC (arm A) versus CVP (arm B) as first line treatment. Patients and method: The patients were randomized into the two treatment arms, 51 patients. The diagnosis of CLL was established according to the criteria of the International Workshop on CLL (IW CLL) 1998. Eligibility criteria were age 65 years, ECOG performance status 0-1 with high risk category (Rai stage III-IV) or Rai stage I-II if they have at least one of the followings: one or more of the disease related symptoms, progressive marrow failure, massive splenomegaly or lymphadenopathy or progressive lymphocytosis > 50% in 2 months or lymphocyte doubling time < 6 months. Arm A: Cyclophosphamide 250 mg/m2 IV. D1 to D3 and Fludarabine 25 mg/m2 D1 to D3. Arm B: Cyclophosphamide 400 mg/m2 IV. D1 to D3, Vincristine 1.4 mg/m2 D1 and Prednisone 100 mg/m2 D1 to D5 P.O. Cycles to be repeated every 21 days. Hematological toxicity was recorded according to the NCI sponsored working
group revised guidelines for diagnosis and treatment of CLL (Cheson et al 1996). Evaluation of response was done according to the NCI-WG response criteria. Patient with stable or progressive disease after the 3rd cycle were excluded from the study while PR and CR cases continued to 6 cycles of the same regimen. Bone marrow biopsy and Immunopheno- notyping were done to confirm the response to treatment. Results. The median follow-up of the whole group was 3 years (Range 33 -65). They included 42 males and 20 females. Twenty cases had stage III and 21 patients for each of stage II and III. The median TLC was 81×10^9/L. The median lymphocyte count was 70×10^9/L. The median hemoglobin level was 9.2 g/dl, while the median platelets count was 150×10^9/L. Pre- treatment bone biopsy showed diffuse pattern in 49 cases (79%) and the median lymphocyte in bone marrow was 85.5%. Complete clinical remission was reported in 15 /31 cases in arm A (48.4%) compared to 6 /31 in arm B (19.4%) p=0.17. Confirmed CR by bone marrow biopsy was reported in 10 cases in arm A (32.3%) and only 3 cases in arm B (9.7%). Focal partial response with nodules (PR-nod.) was reported in 5 cases (16.1%) in arm A and 3 cases (9.7%) in arm B. Median time to disease progression was 25 months in arm A and 6 months in arm B (p=0.03). At 2 years, no significant difference in survival between both arms was detected (83.9% for arm A versus 74.2% for arm B). Conclusion: The combination of FC is able to induce higher response rate with better quality of response at the level of BM biopsy. There was a statistically significant difference in time to disease progression in favor of FC regimen but no significant difference in overall survival.

0792 ORAL FLUDARABINE AND CYCLOPHOSPHAMIDE IN UNTREATED PATIENTS AFFECTED BY CHRONIC LYMPHOCYTIC LEUKEMIA: A PILOT STUDY

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Backgrounds. The introduction of fludarabine into the treatment of chronic lymphocytic leukemia (CLL) has improved the complete remission rate (CR), overall response rate (OR), and progression free survival (PFS) compared with alkylator based regimens. Its synergistic action with cyclophosphamide and its oral route of administration has demonstrated significative advantages as frontline therapy in untreated CLL patients with advanced disease. The oral formulation of fludarabine showed a similar safety profile and response rate as the endovenous compound. Aims. Primary end-point was to test efficacy and safety of the oral formulation of fludarabine combined with cyclophosphamide as front-line therapy of high-risk CLL. As secondary end-point we examined the impact of new prognostic factors associated with progression free survival (PFS). Results. Starting from December 2002, 30 untreated patients with advanced CLL, 20 male and 10 female, with a median age of 68.5 years (52-75) received oral fludarabine (30 mg/m²/day) and oral cyclophosphamide (250 mg/m²) for 3 consecutive days and the oral scheme every 4 weeks, for a total of 6 cycles. At study entry, 25 patients were in stage B-II with progressive disease, 2 in stage C/III, and 3 in stage C/IV. Twelve patients had unmutated and 11 mutated IgVH genes, while in the remaining 7 patients the IgVH gene mutation status was not evaluable. Fifteen patients had more than 20% ZAP-70 positive CLL B-cells, and four patients had the high rish cytogenetic abnormalities del(11)(q23) or del(17)(13.1). Results. Among the 26 evaluable patients, 12 obtained CR (46%), 8 PR (31%). Of the remaining patients 3 had stable disease and 3 progressive disease. In terms of haematological toxicity, 6 patients developed grade IV neutropenia and received G-CSF treatment, while two patients developed severe anaemia (grade III and IV) that required red blood cell transfusions. Only one patient developed a transient febrile neutropenia of unknown origin, but did not require hospitalization. Mild extra-hematological toxicity consisting of nausea and vomiting occurred in six patients during the treatment. No significant differences were noticed in terms of CR and OR rate between the IgVH mutated and unmutated groups (p=ns). Among the 5 patients who have relapsed so far, 4 had unmutated and only 1 had mutated IgVH genes (p=ns), and all three patients that required new treatment (NCI WG criteria) had unmutated IgVH. Conclusion: Oral fludarabine plus cyclophosphamide as front-line therapy in CLL achieved a good overall response rate in our series of patients (46% CR and 31% PR). The haematological and extra-hematological side effects were well tolerated and the oral scheme was easy to administer. The differences in terms of CR, OR and PFS between the IgVH mutated and unmutated groups did not reach statistical significance. However, a longer follow-up is required to define the possible correlation between these prognostic factors and treatment outcome.

0793 CYTOGENETIC CHARACTERIZATION OF B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA / B-CELL SMALL LYMPHOCYTIC LYMPHOMA (B-CLL/B-SLL)

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Background. Chromosome abnormalities studied in a debut of B-CLL/B-SLL provide the important prognostic information. However, inconsistent information about the prognostic significance of cytogenetic aberrations in B-CLL/B-SLL is still present. There are also few data available looking in particular at cytogenetic characterization of the rare subtype of this disease - B-SLL. Aims. To define the type and frequency of chromosome changes of B-CLL/B-SLL and to estimate their prognostic significance. Methods. We studied 135 patients with B-CLL/B-SLL, 90 men and 45 women. The median age was 58 years (range 23 - 84 years). Median follow-up for survival was 32 months. The diagnosis was made by clinical, cytological, histological and immunofenotypic criteria. CD38 expression was measured by flow cytometry in all patients. IgVH mutation status was determined in 61 patients. We performed conventional cytogenetic assay (CCA) and fluorescence in situ hybridization (FISH) using multicolor probe sets LSI p53 / LSI ATM and LSI D13S319/LSI 13q34/CEP12 on mononuclear cells. All B-CLL patients did not receive specific therapy till the moment of cytogenetic analysis. Results. Del1q14 was the most frequent cytogenetic abnormality; it was revealed in 34 (25%) cases. Del11q23 was found in 26 (19%) cases, trisomy 12 in 17 (13%) cases and del19p13 in 8 (6%) cases. Complex karyotype was obtained in 6 (5%) patients. B-SLL was diagnosed in 11 (8%) patients, in 8 (73%) of them we identified del11q23, in 4 (36%)—trisomy 12. We did not reveal chromosome abnormalities in one of these patients. The combinations of del1q23 and trisomy 12 were found in 2 B-CLL patients. The frequency of del1q23 and trisomy 12 in B-SLL patients is higher than in B-CLL patients (p<0.0001 and p=0.02, respectively). There was no one B-CLL patient with del13q14, though it is the most frequent aberration in B-CLL patients. The amount of CD38® tumor cells 30% or more was typical for the majority of B-CLL patients with del1q23 (p=0.02), complex karyotype (p=0.031) and was also identified in all B-SLL patients. The amount of CD38® tumor cells less than 30% was characteristic for patients with del13q14 as a single aberration (p=0.045). 3% or more IgVH mutation was typical (p=0.039) for B-CLL patients without revealed aberrations. The IgVH data were obtained in 4 of B-SLL patients; all of them had less than 3% IgVH mutation. According to the survival rate of B-CLL/B-SLL patients with different karyotype we have allocated 3 prognostic groups of cytogenetic signs: favorable (the absence of aberrations or del13q14 as a single abnormality; the group of intermediate prognosis—patients with del1q23 or trisomy 12; unfavorable—del17p13 or complex karyotype. The overall survival in all 3 patients groups differed each from other, p<0.025 (see figure). Conclusions. For B-CLL normal karyotype and del13q14 as a single aberration are the factors of favourable prognosis, del1q23 and trisomy 12 are the signs of intermediate prognosis, the unfavourable prognostic factors are del17p13 and complex karyotype. Cytogenetic features of B-CLL are del1q23, to a lesser degree - trisomy 12 and the absence of del13q14.
Somatic hypermutation in the variable region of the immunoglobulin heavy chain gene (IgVH) has been shown to be a powerful prognostic parameter in chronic lymphocytic leukaemia (CLL) and has the capacity to differentiate between two disease subsets. In recent years individual IgVH gene rearrangements, in particular VH3-21, have been shown to define unique disease entities in CLL. Aims. To determine the most commonly expressed VH genes in a Northern Irish cohort of CLL patients and to ascertain if variations in gender, light chain restriction and the presence of chromosomal abnormalities show associations with IgVH mutational subgroups and individual VH gene rearrangements. Methods. Two hundred and twenty-eight CLL patients were recruited from Belfast City Hospital and surrounding hospitals. IgVH mutational status and VH gene usage were determined using standard BIOMED-2 primers and protocol followed by sequence analysis. FISH analysis was performed to identify the presence of recurrent chromosomal aberrations. Light chain restriction was determined by standard immunophenotyping techniques. Results. The most common VH gene rearrangements were VH4-34 (13.5%), VH1-69 (12.3%), VH1-2 (7.9%), VH3-21 (7.9%) and VH1-3 (7.5%). Females showed a bias towards mutated IgVH status (2M: 1UM), overuse of VH4 genes (40%) and a lower frequency of trisomy 12 (7%). In contrast males showed no mutational bias (1:1), overrepresentation of VH1 genes (38%) and a higher incidence of trisomy 12 (21%). VH3-21, VH3-48 and VH3-53 showed preferential lambda light chain restriction, while VH1-69 had overrepresentation of kappa light chains. Further analysis of VH3-48 and VH3-53 gene usage showed a preponderance of females (7:1, 3:1 respectively), lambda light chain restriction and inferior outcome irrespective of IgVH mutational status. Conclusions. This study has demonstrated gender related differences in CLL which can be explained partly by the increased incidence of mutated IgVH genes, biased use of VH4 genes and lower frequency of adverse chromosomal aberrations in female patients. This study confirms that gender related survival differences exist in CLL patients and that gender should be taken into account in risk stratification of patients at presentation. Furthermore, specific VH gene usage is associated with have distinctive characteristics, supporting the concept that antigens have an important role to play in the aetiology of CLL.
EFFICACY OF RITUXIMAB IN HAIRY CELL LEUKEMIA

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Backgrounds. Hairy cell leukemia (HCL) is a rare B-chronic lymphoproliferative disorder with an indolent course. First-line treatment modalities include 2-chlorodeoxyadenosine, 2-deoxycoformycin and interferon-α. The efficacy of anti-CD20 (Rituximab) in other BCLDs and the strong expression of CD20 by hairy cell leukemia lymphocytes indicate that Rituximab could be an alternative in the treatment of HCL. The experience of a single Hematology Unit in the treatment of relapsed HCL with Rituximab. PATIENTS AND METHODS. We retrospectively analyzed all hairy cell leukemia patients who received Rituximab as salvage therapy in 1st or subsequent relapse. The clinical and laboratory characteristics at diagnosis and relapse, primary treatment and its duration, as well as response to treatment were analyzed. Results. 10 patients treated with Rituximab were located among 110 patients diagnosed with HCL between 1980 and 2005. 9 were males and their median age at diagnosis was 46 years (range: 42-94). All but two patients presented with splenomegaly with a median spleen size of 8.5 cm below left costal margin (range: 2-30 cm). 9/10 patients were leukopenic at diagnosis with the remaining one showing lymphocytosis. All patients displayed a typical immunophenotype from blood and/or bone marrow (CD20 strongly+, CD23−, CD19+, CD22+, FMC-7+, CD11c+, CD25−, CD103−). Four of them were CD25+ and two CD110+. Six patients received Rituximab at 1st relapse. Among them, one had received 2-deoxycoformycin as 1st line treatment, one 2-chlorodeoxyadenosine and four interferon-α as induction and maintenance. 3 patients had received more than one prior treatment. Two of them were IFN-α resistant and one had discontinued IFN-α due to side-effects. One patient received Rituximab at diagnosis, due to older age and possible complications with other therapies. The median time from diagnosis to Rituximab initiation for the 9 patients was 61 months (range: 19-168). Rituximab was administered at 375 mg/m2 weekly for 6 cycles. Overall response rate was 77%. 2 patients went into complete remission, including a negative bone marrow biopsy, a negative immunophenotype and a negative immunoglobulin gene rearrangement. 2 patients achieved a complete hematologic remission with normalization of their cytopenias, but with remaining bone marrow infiltration of <25%. 3 patients achieved a partial hematologic response, while 1 patient was Rituximab resistant. One patient is not evaluable and the remaining one discontinued treatment after the first cycle due to the development of thrombocytopenia that was attributed to the drug. No other complications were recorded, except of mild infusion-related symptoms. Among the complete hematologic responders, no patient has relapsed with a median follow-up of 12 months (range: 4-36). Among partial responders, one achieved a complete response including a negative bone marrow and immunophenotype after Rituximab retreatment, one is alive in partial remission without the need of further therapy and one progressed within 5 months from Rituximab administration. Conclusions. Rituximab is a highly effective treatment for HCL in relapse with a response rate of 77%. Retreatment with Rituximab, or maintenance may be important, since ongoing responses are seen.

SUCCESSFUL AND COST-EFFECTIVE PROPHYLAXIS AND TREATMENT OF TUMOR LYYSIS SYNDROME (TLS) WITH LOW DOSES OF RASBURICASE

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Backgrounds. TLS commonly occurs in patients with hematologic malignancies and is characterized by elevation of uric acid, potassium and phosphate and by hypocalcemia. A major complication is renal failure caused by precipitation of uric acid and/or calcium phosphate crystals. Treatment consists of hydration, correction of electrolyte disturbances and lowering of uric acid. Rasburicase, a recombinant urate oxidase, has proven to be highly effective in lowering serum uric acid levels, and its application is not restricted in patients with renal failure. Costs for a full seven-day course of rasburicase in the dosage recommended are high and amount to approximately 4800 €. For rasburicase a 86% reduction of uric acid levels 4 hours after the first dose has been reported. Therefore, the question arose whether lower doses of rasburicase than those recommended by the manufacturer can be applied for efficient prophylaxis and treatment of TLS. Methods. 40 patients (27 male, median age 65 yrs, range 16-88) received rasburicase in low doses for prophylaxis or treatment of TLS. Ten patients had acute leukemia, 6 had myeloproliferative disorders, 5 patients had high-grade Non-Hodgkin’s lymphoma, 15 had low-grade Non-Hodgkin’s lymphoma and 4 patients had solid tumors. TLS was classified into laboratory and clinical TLS and graded according to Cairo and Bishop. Thirty-five patients had elevated serum creatinine levels, the median creatinine level in these patients was 2.65 mg/dL (1.18-8.61 mg/dL). Laboratory TLS was diagnosed in 5 patients, 28 patients had clinical TLS. Results. Seven patients received rasburicase for prophylaxis of TLS. The mean LDH level in this group was 706 U/L, the mean uric acid level was 11.7 mg/dL before application of rasburicase. The median number of doses of rasburicase applied was 2 (range 1-2), the median total dose was 3 mg (0.052 mg/kg). After application of rasburicase the mean uric acid level decreased by 66% and was 3.7 mg/dL. None of the patients developed TLS. Thirty-three patients received rasburicase for treatment of TLS. The mean LDH level was 1668 U/L. The mean uric acid level was 15.8 mg/dL before application of rasburicase. The median number of doses of rasburicase applied was 1 (range 1-5), the median total dose was 3 mg (0.058 mg/kg). After application of rasburicase the mean uric acid level decreased by 80% and was 3.2 mg/dL. No patient required renal replacement therapy. Rasburicase was well tolerated by all patients without side effects. Conclusion. We applied rasburicase doses as low as 3,2-4,3% of the dose recommended by the manufacturer. Rasburicase applied in low doses proved to be effective for prophylaxis and treatment of TLS, even in patients with renal failure. For some patients doses as low as one vial of 1.5 mg of rasburicase was sufficient to control hyperuricemia, lowering the costs to 83. Cost-effective treatment becomes an increasingly important issue regarding limited budgets in health care. Further studies have to be conducted to establish dosing regimens for different clinical settings.
Despite moderate therapeutic advances in recent years, improvement of each drug combinations at baseline was 59.7%, 60.0%, and 61.9%, in the reference costs. Health outcome measures included time to progression and overall survival at week 12 and baseline to week 24 were significant for all parameters: FWB (p=0.005); FATS (p<0.001); NEATS (p<0.001); ANS (p<0.001). Mean baseline LSAE scores ranged from 46.5/50.4 mm (normal: 70/100 mm) indicating poor QOL at study initiation. Mean changes in LSAE at week 12 were clinically and statistically significant: energy level, 8.9 mm (p<0.001); daily activities, 7.5 mm (p<0.001); overall QOL, 6.8 mm (p<0.001); improvements were even greater at 24 weeks for all parameters. Both FACT-An and LSAE scores improved as Hb level increased, with increases >2 g/dl demonstrating the greatest mean improvements at week 12 and 24. Baseline mean Hb for combined diagnostic groups was 10.4 g/dl, patients with MM had the lowest mean Hb (10.0 g/dl) and patients with HD had the highest mean Hb (10.8 g/dl). Mean Hb increases after 3-5, 12, and 24 weeks of epoetin alfa treatment were 1.0 g/dl, 1.7 g/dl, and 1.7 g/dl, respectively (p<0.001 at all time points). MM patients had the greatest Hb increase at week 12 (2.1 g/dl). Transfusions were administered to 25% of patients during the study. Conclusions. Treatment with epoetin alfa resulted in improved QOL in anemic patients with HD, NHL, CLL, and MM. This improvement was associated with increases in Hb attained with epoetin alfa 40,000 IU QW.

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Backgrounds. Despite moderate therapeutic advances in recent years, improvement of each drug combinations were outcome measures (lenalidomide) is a new immunomodulatory drug, which is supplied in named patient programs (NPP) in relapsed or refractory MM patients in Europe, including the UK. We conducted a preliminary assessment of the cost-effectiveness of lenalidomide from the perspective of the UK’s National Health Service (NHS). Methods. A comprehensive health economic decision analytic model was developed to evaluate and compare the costs and health benefits of lenalidomide plus dexamethasone versus bortezomib. The model combined data from three large-scale randomized clinical trials: two studies compared bortezomib with high-dose dexamethasone alone (APEX study, n=699 patients). The model also built on published literature on additional parameter estimates and was validated by two UK hematologists. The model incorporated direct medical costs related to drugs, drug administration, diagnostic tests, office visits and other medical resource use associated with standard care and complications due to disease or adverse events. Unit cost estimates were based on NHS Trust/Primary Care Trust (PCT) National Reference Costs. Health outcome measures included time to progression and overall survival at week 12 and baseline to week 24 were significant for all parameters: FWB (p=0.005); FATS (p<0.001); NEATS (p<0.001); ANS (p<0.001). Mean baseline LSAE scores ranged from 46.5/50.4 mm (normal: 70/100 mm) indicating poor QOL at study initiation. Mean changes in LSAE at week 12 were clinically and statistically significant: energy level, 8.9 mm (p<0.001); daily activities, 7.5 mm (p<0.001); overall QOL, 6.8 mm (p<0.001); improvements were even greater at 24 weeks for all parameters. Both FACT-An and LSAE scores improved as Hb level increased, with increases >2 g/dl demonstrating the greatest mean improvements at week 12 and 24. Baseline mean Hb for combined diagnostic groups was 10.4 g/dl, patients with MM had the lowest mean Hb (10.0 g/dl) and patients with HD had the highest mean Hb (10.8 g/dl). Mean Hb increases after 3-5, 12, and 24 weeks of epoetin alfa treatment were 1.0 g/dl, 1.7 g/dl, and 1.7 g/dl, respectively (p<0.001 at all time points). MM patients had the greatest Hb increase at week 12 (2.1 g/dl). Transfusions were administered to 25% of patients during the study. Conclusions. Treatment with epoetin alfa resulted in improved QOL in anemic patients with HD, NHL, CLL, and MM. This improvement was associated with increases in Hb attained with epoetin alfa 40,000 IU QW.


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Backgrounds. Anemia from disease and/or chemotherapy is a common complication in patients with Hodgkin’s disease (HD), non-Hodgkin’s lymphoma (NHL), chronic lymphocytic leukemia (CLL), and multiple myeloma (MM). Because anemia is associated with fatigue and other symptoms of diminished quality of life (QOL), treatment of anemia in patients with hematologic malignancies is important. Aims. The primary objective was to compare change in QOL from baseline to week 12 when anemia is corrected with a once-weekly (QW) regimen of epoetin alpha in patients with HD, NHL, CLL, or MM receiving chemotherapy. Improvements in Hb levels and transfusion requirements were also reported. Methods. EPOLYM was an international, multicenter, open-label, Phase IIIb, 24-week trial in anemic (hemoglobin [Hb] <12 g/dL) cancer patients receiving chemotherapy. Patients received epoetin alpha 40,000 IU QW subcutaneously to a target Hb of 11.5-13.0 g/dL with dosage adjustments based on clinical response. The primary objective of the study was evaluated by the Functional Assessment of Cancer Therapy-Aemia (FACT-An) which measured functional well-being (FWB), fatigue subscale score (FATS), non-fatigue subscale score (NEATS), and total anemia subscale score (ANS). The Linear Analog Scale Assessment (LASA; also known as the Cancer Linear Analog Scale [CLAS]) for energy level, daily activities, and overall QOL was also employed. Statistical significance was analyzed by the paired sample t-test. Changes in Hb level and transfusion requirements from baseline were evaluated. Results. Intent-to-treat population was 1034 patients: 416 NHL, 307 MM, 165 HD, and 145 CLL (1 unspecified). Epoetin alpha dosage was increased for 57.8% of patients. Mean baseline FACT-An scores were: FWB 14.9; FATS 31.3; NEATS 19.4; and ANS 50.7. Mean increases for FACT-An from baseline to week 12 and baseline to week 24 were significant for all parameters: FWB (p=0.005); FATS (p<0.001); NEATS (p<0.001); ANS (p<0.001). Mean baseline LSAE scores ranged from 46.5/50.4 mm (normal: 70/100 mm) indicating poor QOL at study initiation. Mean changes in LSAE at week 12 were clinically and statistically significant: energy level, 8.9 mm (p<0.001); daily activities, 7.5 mm (p<0.001); overall QOL, 6.8 mm (p<0.001); improvements were even greater at 24 weeks for all parameters. Both FACT-An and LSAE scores improved as Hb level increased, with increases >2 g/dl demonstrating the greatest mean improvements at week 12 and 24. Baseline mean Hb for combined diagnostic groups was 10.4 g/dl, patients with MM had the lowest mean Hb (10.0 g/dl) and patients with HD had the highest mean Hb (10.8 g/dl). Mean Hb increases after 3-5, 12, and 24 weeks of epoetin alfa treatment were 1.0 g/dl, 1.7 g/dl, and 1.7 g/dl, respectively (p<0.001 at all time points). MM patients had the greatest Hb increase at week 12 (2.1 g/dl). Transfusions were administered to 25% of patients during the study. Conclusions. Treatment with epoetin alfa resulted in improved QOL in anemic patients with HD, NHL, CLL, and MM. This improvement was associated with increases in Hb attained with epoetin alpha 40,000 IU QW.
conducted to test the robustness of the assumptions. Results. The mean total cost of FluCam and FCR treatment was estimated at approximately 26,426 and 35,324, respectively, assuming 6 cycles of therapy. The ORR reported for FluCam is 83% and for FCR it is 73%, which would yield a cost per responder of 31,838 and 48,389, respectively. Data on response duration for either therapy is currently not available, but preliminary estimates of time to progression (TTP) are 15 months for all patients and for responders assuming a base-case scenario of a 73% ORR and 24 months of response duration for each therapy, then the expected number of months in remission per patient treated would be 17.52 months, or 1.46 years. Comparing costs and assuming a similar level of clinical benefit, the cost per year in remission for FluCam and for FCR would be 18,100 and 24,194, respectively. Sensitivity analyses suggest that FCR would need to be at least 25% more effective than FluCam to achieve equivalence in terms of cost-effectiveness, which is unlikely given that alemtuzumab is more effective than rituximab as a monotherapy. Conclusions. Based on this analysis, FluCam is potentially a more cost-effective treatment strategy for relapsed/refractory CLL. The findings of this analysis imply that for each relapsed CLL treatment where FluCam is used rather than FCR, the cost savings to the treasury will be on average 28,898 if both therapies were equivalent in terms of efficacy, and even more if FluCam is the more effective alternative. Randomized trials comparing the effectiveness and cost of both combinations are needed to confirm the findings of this analysis.

0802
PAIN IN HAEMATOLOGY: AN OUTCOME RESEARCH PROJECT TO EVALUATE THE EFFECTIVENESS OF TREATMENT IN IN-HOSPITAL PATIENTS
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Background. Management of pain associated with blood-related diseases is often difficult and inadequate, notwithstanding the availability of several therapeutic interventions and of well-known guidelines and protocols. Patients and Methods. A six-months multicenter study involving the wards of three Italian haematological Centres was started in October 2005 to investigate the epidemiology and the clinical characterisation of pain in haematological patients. A treatment protocol based on the cancer pain WHO analgesic ladder was applied. Data reported as January 2006 in the 286 patients with a median age of 67 (19-93) years. The total number of admissions during the study period was 415. Haematological diagnoses were as follows: 85 (20%) non-Hodgkin’s lymphomas, 55 (20%) acute myeloblastic leukaemia (AML), 29 (10%) multiple myeloma (MM), 16 (5%) acute lymphoblastic leukaemia (ALL), 9 (3.5%) other lymphoproliferative disorders, 26 (9%) myelodysplastic and chronic myeloproliferative disorders and 46 (16%) non-malignant diseases. Results. Of 286 patients, 128 (45%) experienced almost one pain syndrome, for a total of 174, which pathophysiology were diagnosed as follows: 76 (45.6%) deep somatic, deriving from the bone in most cases, 34 (19.5%) superficial somatic (mucositis and cutis derangements), 30 (17.2%) visceral, 15 (8.6%) neuropathic, 15 (8.6%) mixed or by unknown mechanisms and 4 (2.2%) iatrogenic or infection related. A diagnosis of MM, ALL and AML, and an advanced disease phase were significantly associated with a higher incidence of pain. The treatment protocol was based on three steps, according to the intensity of pain. The first step (mild pain) included paracetamol, the second step (moderate pain) treatment of the third step (severe pain) morphine. In selected cases, oxycodone or fentanyl patches were used in the place of morphine to treat severe pain. Of 174 pain syndromes, 48 (28%), 49 (29%) and 77 (53%) pain syndromes were treated with a first-line therapy according to the first, the second and the third step respectively. Of the 97 pain syndromes treated according the first two steps, 37 (38%) needed a treatment escalation to the third step after 4 (1-12) days. No serious adverse effects were recorded. The adopted treatment approach, integrating causal interventions (if applicable) and analgesic measures, including the adjuvants to treat neuropathic pain, allowed a prompt relief in more than 90% of the pain syndromes. No serious adverse effects were recorded. Conclusion. These preliminary results indicate that, in the setting of haematological wards, pain is a significant symptom requiring prompt medical attention. Moreover, our data outline that most pain syndromes can be effectively controlled by the currently available treatment strategies. Therefore, the implementation of clinical pathways and standardized protocols based on well-defined algorithms can provide the auspice advancements toward a ‘pain-free’ haematological hospital.

0803
COST-EFFECTIVENESS OF ‘Y-IBRITUMOMAB TIUXETAN (Y-ZEVALIN) VERSUS RITUXIMAB MONOTHERAPY IN PATIENTS WITH RELAPSED FOLLICULAR LYMPHOMA
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Backgrounds. Currently, there are limited cost-effectiveness data comparing the use of 90Y-Zevalin with rituximab in follicular lymphoma (FL). The primary objective of this analysis was to estimate the (incremental) cost-effectiveness of a single dose of 90Y-Zevalin 0.4 mCi/kg compared with: 1) standard rituximab treatment of 375 mg/m² weekly for 4 weeks (4-dose rituximab); and 2) standard rituximab followed by 4 weeks of maintenance therapy (8-dose rituximab) in patients with FL. Methods. The effectiveness data used for the analysis were derived from the only 2 clinical studies published to date enrolling comparable populations where patients had received either ‘Y-Zevalin or rituximab monotherapy. These were a randomized trial of ‘Y-Zevalin versus 4-dose rituximab,
The mean total cost of treatment with 4 doses of rituximab was estimated to be lower at EUR 9,847, whereas the cost associated with an 8-dose rituximab treatment was EUR 20,112. In terms of health benefits, the average number of disease-free months per patient treated was highest for 90Y-Zevalin at 14.4 months followed by 11.4 months for the 8-dose rituximab and 6.2 months for the 4-dose rituximab. When the estimates of health benefit are combined with costs, the analysis demonstrates a mean cost per disease-free month for 90Y-Zevalin of EUR 1,272, the lowest of the 3 therapies, followed by EUR 1,599 for 4-dose rituximab, and EUR 1,770 for 8-dose rituximab.

**Conclusions.** The findings imply that for each third-line follicular NHL treatment with 90Y-Zevalin is used rather than 4-dose rituximab, the additional cost to the payer would be, on average, EUR 8,426. For this additional cost, the benefit to the patient would be an average 8.2 additional months in remission, over and above what would have been gained with 4-dose rituximab therapy. Furthermore, when the costs and benefits of Y-Zevalin are compared with those of the 8-dose rituximab regimen, Y-Zevalin is the more cost-effective strategy.

**0804**

**COST-EFFECTIVENESS OF ONCE-DAILY ORAL CHELATION THERAPY WITH DEFERASIROX VERSUS INFUSIONAL DEFEROXAMINE IN TRANSFUSION-DEPENDENT THALASSEMIC PATIENTS: A BRAZILIAN PERSPECTIVE**

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**Background.** Transfusion-dependent thalassemic patients require chelation therapy to treat iron overload and prevent its complications including cardiac, endocrine and hepatic toxicity. Deferoxamine (DFO) is an effective chelator but must be administered as an 8-12-hour infusion 5-7 times per week, leading to poor compliance and/or reduced quality of life. Deferasirox (DSX) is a novel once-daily oral iron chelator with a high iron-binding potency and selectivity that has recently been approved by the FDA, Swissmedic and ANVISA in Brazil. Clinical studies with DSX have shown superiority for the treatment of transfusional iron overload. Cost-effectiveness analysis is a technique used to determine whether the benefits of new therapies are worth their additional costs. The objective of this analysis was to evaluate, from a Brazilian perspective, the cost-effectiveness (CE) of DSX vs DFO in patients with transfusion-dependent thalassemia. Methods. A decision-analysis model was used to estimate the total additional lifetime costs and quality-adjusted life expectancy (QALY) gained with DSX versus DFO in patients with transfusion-dependent thalassemia (≥28 transfusions per year). Compliance patients were assumed to receive doses of DSX and DFO that have been shown to be similarly effective in such patients. Probabilities of complications of iron overload and death by average compliance with chelation were estimated using data from published studies. Compliance with DFO was based on analyses of US health insurance claims data of transfusion-dependent thalassemic patients. Cost of cardiac disease was taken from a published local study in the public healthcare system. The costs of other complications of iron overload conservatively were not included in the model due to lack of local data. Because data on compliance with DSX in typical clinical practice are unavailable, we used published data on compliance with the three-times-daily oral chelator deferiprone vs DFO. Utilities (weights representing patient quality of life) were obtained from a study that used time-trade-off techniques to measure patient preferences for oral vs intravenous chelation therapy. The analyses were based on the anticipated cost of DSX and the current DFO cost for public payers excluding taxes. The administration cost of DFO was calculated from the patient perspective using the Brainside Table for syringes, needles, scalp and other materials. For the standard infusion pump (Infusa T Medis) was used the price to consumer from a local distributor. Results were generated for DFO-naive patients (age 2 years, no prior DFO therapy). Costs and QALYs were discounted at 3% annually. Costs were reported as 2006 US dollars. Results. The cost of DFO administration was $195 per month representing 22% of the cost of chelation therapy with DFO. In DFO-naive patients, DXS results in a gain of 3.8 QALY vs DFO. These results were obtained at an expected discounted lifetime cost of $90,515 per patient. CE is $23,425 per QALY gained. Conclusions. Assuming a cost-effectiveness threshold of less than three times the GDP per capita (WHO, 2002) and that the GDP per capita in Brazil is $5,500 (2005), deferasirox is a cost-effective use of the healthcare resources in patients with transfusion-dependent thalassemia.

**0805**

**COST EFFECTIVENESS OF ADDING IMATINIB TO CHEMOTHERAPY IN ADULT PATIENTS WITH PHILADELPHIA CHROMOSOME-POSITIVE ACUTE LYMPHOCYTIC LEUKAEMIA: AN EXPLORATORY ANALYSIS**

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**Backgrounds.** The prognosis of Philadelphia chromosome-positive acute lymphoblastic leukaemia (Ph+ALL) in adults is extremely poor. Imatinib has been reported to successfully induce and consolidate remissions and extend disease-free survival in Ph+ ALL patients with good tolerability in clinical studies. Aims. This study explores the cost effectiveness of imatinib plus conventional chemotherapy regimens v. conventional chemotherapy alone. Methods. A Markov model was used to follow a hypothetical cohort of 1000 adult Ph+ALL patients receiving imatinib plus conventional chemotherapy (CC) or CC alone. Patients were modeled for a total of ten years in monthly intervals. Patients were distributed over time into three health states: alive without disease progression (DFP), alive with disease progression (DS), or dead. For the purpose of this model, patients were assumed to continue imatinib therapy (900 mg daily) for up to two years as long as they remained in the DFP state. Probabilities of being in the states were derived from the published literature of case series for patients with Ph+ALL receiving either treatment. In the absence of relevant data pertaining to Ph+ALL, assumptions about costs and utilities were derived from a cost analysis of chronic myeloid leukemia. Only direct medical costs were included in the analysis using a U.S. health care payer perspective. All outcomes were discounted at a 3% rate per annum. Results. Based on the literature, the median disease free survival and overall survival of Ph+ALL adult patients with CC were 6 and 9 months (Thomas X et al. 1999), respectively. The 12-month disease free survival and survival for imatinib+CC were 72% and 84% (Towatari 2004), respectively. Total discounted survival was 1.02 years for CC and 4.29 years for imatinib+CC. Total discounted disease free survival was 0.76 year for CC and 2.79 years for imatinib+CC. Assuming utility weights of 0.854 and 0.596 for DFP and DS, respectively, the total discounted quality adjusted lifetime (QALY) were estimated to be 0.88 and 3.88 for CC and imatinib+CC, respectively. Thus, the net incremental gain in discounted quality adjusted survival was 2.47 QALYs. Total incremental treatment costs for imatinib+ CC were $102,507 as compared to CC over 10 years. Therefore, the incremental cost per QALY of imatinib+CC vs CC alone was approximately $41,500 (i.e., $102,507 divided by 2.47 QALYs) which is within the range of usual acceptable cost effectiveness threshold. Conclusions. For adult ALL patients with poor prognosis due to Ph+ALL, our exploratory analysis suggests that, given the underlying data and assumptions, adding imatinib to current chemotherapy regimens may be cost-effective compared to chemotherapy alone.
To ascertain the feasibility of neonatal cord blood screening in the Sultanate of Oman in an effort to determine the prevalence of haemoglobinopathies by a cost-effective method. 

Methods. A total of 1575 cord blood samples were screened for presence of possible haemoglobinopathies by high performance liquid chromatography (HPLC) technique using Biorad Variant™ program between April 2005 & February 2006. Complete blood counts were also obtained on Cell Dyn 4000 automated blood cell counter. All samples were then processed to isolate and store mononuclear leukocytes for subsequent molecular diagnostics. Results. The findings indicated a 37.21% incidence of α-thalassaemia [predominantly - αβ7/αα]. Furthermore, the overall incidence of other haemoglobinopathies was 10.36% with 6.45% and 2.36% incidence of sickle haemoglobin and β-thalassaemia respectively. On HPLC, D-window, E-window and C-window were present in 1.11%, 0.33% and 0.13% of the samples respectively, with a few samples presenting with unknown peaks that need further studies. 

Summary/Conclusions. The wealth of information obtained by screening highlights the significantly high incidence of haemoglobinopathies in newborns in the Sultanate of Oman and emphasizes the value of neonatal cord blood screening to be implemented as the first step in the national strategy towards prevention of haemoglobinopathies. Although cord blood screening cannot give a definitive diagnosis it can identify the small group of neonates that require further testing. Moreover, the cost of testing per sample was approximately 1 Euro.

Figure 1. Prevalence of hemoglobinopathies in the newborn.

**0808**

VALUE OF TRANSFUSION-FREE LIVING IN MDS: RESULTS OF HEALTH UTILITY INTERVIEWS WITH PATIENTS

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Backgrounds. Achieving transfusion independence in patients with transfusion-dependent MDS has been defined as a key treatment goal in clinical trials of new interventions and in everyday clinical practice as new treatments have become available. However, data is lacking on how patients themselves value health states associated with transfusion-independence as opposed to transfusion-dependence. Methods. We performed health utility interviews with MDS patients in the US, France, and the UK to elicit the value of transfusion-independence or reduced transfusion burden compared to transfusion dependence (i.e., three distinct health states). Health state descriptions were developed based on literature and reports from MDS patient focus group discussions, and were validated by a haematologist. Each health state card included different severity/intensity of problems on the following quality-of-life (QoL) domains: reliance on blood transfusions and health care provider facility; need to arrange one’s life around medical appointments; fatigue and tiredness that limits performance of routine physical activities; interference of disease with social and family life; worry about the future due to health condition; discomfort associated with medical conditions and treatment, and the feeling of being at risk of infection; reliance on support persons for self care and routine activities; feelings of being a burden to family; and feeling sad, hopeless, and helpless. Face-to-face interviews were conducted with the Feeling Thermometer Visual Analogue Scale (VAS) and the Time Trade-off (TTO) method to value the health states on a scale anchored on 1 (perfect health) and 0 (dead). We administered background questionnaires on socio-demographic, clinical, and QoL (EQ-5D) characteristics to describe the patient sample. Results. Thirty-eight MDS patients in the US (n=21), France (n=9), and UK (n=9) completed the interview. The mean age was 66 years (range: 29-83), 55% were male. The majority were retired (66%), had secondary/high school education (38%) or higher (24%), and were living with family, a partner or spouse, or friends (76%). The mean time from MDS diagnosis was 5 years (range: 1-25). The majority of patients received blood transfusion(s) previously (87%), and 47% had received a blood transfusion in the last three months. Mean EQ-5D utility score was 0.78, and patients reported at least some problem with mobility (44%), usual activities (39%), pain/discomfort (47%), and anxiety/depression (29%). One patient reported problems with self-care. Few patients had difficulty understanding the rating scale (n=3) and TTO (n=3) exercise. The health utility score for the transfusion-independent health state was significantly higher than for health states with reduced transfusion requirement (0.85 vs. 0.77, p<0.001), and transfusion dependence (0.85 vs. 0.62, p<0.001). Three patients valued transfusion dependence as worse than being dead. Corresponding rating scale scores were 78 vs. 87 (p<0.001), and 78 vs. 32 (p<0.001), respectively. Conclusions. These results show that patients associate a high value with achieving transfusion independence, which, in turn, suggests an important role for new treatments aiming to achieve greater transfusion independence in MDS.

**0809**

HEALTH RELATED QUALITY OF LIFE IS COMPROMISED IN PATIENTS WITH IRON OVERLOAD RECEIVING INFUSION TREATMENT: US AND UK SF-36 AND CHQ SCORES COMPARED TO MATCHED POPULATION NORMS

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Backgrounds. Patients with thalassaemia, sickle cell disease (SCD), and myelodysplastic syndrome (MDS) require blood transfusions as supportive care. One consequence of regular blood transfusions is iron overload (IO) which, if left untreated, will result in morbidity and earlier mortality. Current infusion iron chelation therapy (ICT) requires 8-12 hour infusions, 5-7 days per week, potentially inhibiting adherence and limiting health-related quality of life (QoL) in patients already limited by thalassaemia, SCD, and MDS. Aims. To assess health status of patients on infusion ICT by comparing the Medical Outcomes Study Short Form Health Survey (SF-36) and Child Health Questionnaire (CHQ) responses from individuals with thalassaemia, SCD, and MDS, undergoing infusion ICT against US and UK norms. Methods. Patients with TLA; SCD,
or MDS, currently undergoing infusion ICT, completed the SF-36 and CHQ. In total, 79 participants were assessed (US: thalassemia n=41 and SCD n=9; UK: thalassemia, n=11 SCD n=14, and MDS n=4). The HRQoL instruments, the SF-36 and CHQ, were scored (0 to 100, with higher scores indicating higher QoL) and compared to available age- and/or gender-matched published norms for UK and US. In addition, utilities were estimated for the UK population using published algorithms and compared to population, Florham Park, USA.

Impacts Emotional. Reducing the burden of treatment on the patient by introducing an effective, well tolerated, and more convenient therapy would provide a significant benefit to patients suffering from thalassemia, SCD, or MDS currently undergoing infusion ICT was conducted in 29 patients (11 from the USA; 18 from the UK). Specifically, point differences between UK male norms and UK male study population indicated a decrement ranging from 1.4 for the Mental Health domain to 61.25 for Role Performance domain. Similar results were reflected in the female group in whom, compared to UK norms, point differences were lower and ranged from 5.03 for Mental Health domain to 56.73 points in Role Performance. CHQ data revealed similar results. In the US, compared to age-matched norms, study participants scored lower on all SF-36 domains (decremental point differences ranged from 2.08 for Physical Functioning to 26.64 for Parent-Impact Emotional) except Family Cohesion, General Behaviour, and Self-Esteem. In the UK, compared to age-matched norms, study participants scored lower on all SF-36 scales (reduced point difference ranged from 1.65 for Self Esteem to 33.03 for Parent-Impact Emotional) except Family Cohesion. Differences of these magnitudes are generally considered clinically significant. Further, UK study participants produced a utility score of 0.61. Summary/Conclusions. Results indicated that patients with thalassemia, SCD, or MDS currently undergoing infusion ICT showed much lower HRQoL scores compared to population, and particularly for General Health, Role Physical, and Parent-Impact Emotional. Reducing the burden of treatment on the patient by having an effective, well tolerated, and more convenient therapy would contribute to improving the quality of life of these patients.

0810
ESTIMATED TOTAL ANNUAL COSTS OF INFUSED IRON CHELATION THERAPY IN THE UNITED KINGDOM
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Backgrounds. Patients suffering from β-thalassemia, sickle cell disease, and myelodysplastic syndrome undergoing chronic blood transfusions are at risk for iron overload which, if not treated by iron chelation therapy (ICT), can create serious organ damage and reduce life expectancy. Deferoxoxima (DFO) is the standard of care for the depletion of excess bodily iron. It has to be infused for 8-10 hours, 5-7 times a week. Although the clinical need for ICT is clearly established, less is known about the economic burden of DFO treatment. Aim. To estimate the total annual costs of ICT from the National Health Service's perspective. Two generic HRQoL questionnaires, EuroQol (EQ-5D) and Short Form 36 (SF-36) have been used, higher score corresponding to better quality of life. Results. None of the 19 enrolled patients, aged 25-58 years (mean=33.2) developed inhibitors. Patients reported a mean of 2.37 events/patient/month during ODP (median=1.67, range: 0.5-15) vs. 0.48 events/patient/month during PP (median=0.25, range: 2.5) (Wilcoxon signed test p<0.001). Mean FVIII cost during ODP was 16,149 IU/patient/month during ODP (range: 2,000-50,000) and 26,342 IU/patient/month during PP (range: 16,667-38,333). Mean FVIII consumption was 16,149 IU/patient/month during ODP (range: 2,000-50,000) and 26,342 IU/patient/month during PP (range: 16,667-38,333). The mean cost to treat one bleed was 3,958 (median: 2,391). The incremental cost-effectiveness ratio, i.e. the cost for avoided bleed, was 4,675. At the end of the follow-up period, SF-36 showed a statistically significant improvement in patients quality of life in all domains (p<0.05). Concerning the Physical Component Summary score (PCS) and the Mental Component summary score (MCS), patients on PP showed better results than those on ODP, although no significant difference was found for MCS. Results obtained with EuroQol-5D were comparable to those shown by SF-36, with significantly different Visual Analogue Scale scores after ODP vs. after PP (p<0.05). Summary/Conclusions. These findings showed prophylaxis with RefactoTM dose of 25 IU/Kg tiw in adults with haemophilia was effective and safe. Our cost-effectiveness results can represent the point of reference for other similar evaluations. Furthermore prophylaxis has provided a significant improvement in HRQoL and it should therefore be considered in a cost utility evaluation.

0812
COST UTILITY ANALYSIS OF DEFERASIROX VERSUS DEFEROXAMINE FOR PATIENTS REQUIREING IRON CHELATION THERAPY IN THE UNITED KINGDOM
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Backgrounds. Patients suffering from β-thalassemia (β-thal), sickle cell disease (SCD), and myelodysplastic syndrome (MDS) require lifelong blood transfusions and are at risk of iron overload. If they are not treated with iron chelation therapy (ICT), they can suffer serious organ damage and reduced life expectancy. Desferal, infused subcutaneously for 8 hours per day, 5-7 times per week, is the standard of care for the depletion of excess bodily iron. Exjade is a new once daily oral iron chelation in UK treatment centers and the medical literature. Results indicated that patients with thalassemia, SCD, or MDS currently undergoing infusion ICT showed much lower HRQoL scores compared to population, and particularly for General Health, Role Physical, and Parent-Impact Emotional. Reducing the burden of treatment on the patient by having an effective, well tolerated, and more convenient therapy would contribute to improving the quality of life of these patients.

0810 ESTIMATED TOTAL ANNUAL COSTS OF INFUSED IRON CHELATION THERAPY IN THE UNITED KINGDOM
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Backgrounds. Patients suffering from β-thalassemia, sickle cell disease, and myelodysplastic syndrome undergoing chronic blood transfusions are at risk for iron overload which, if not treated by iron chelation therapy (ICT), can create serious organ damage and reduce life expectancy. Deferoxoxima (DFO) is the standard of care for the depletion of excess bodily iron. It has to be infused for 8-10 hours, 5-7 times a week. Although the clinical need for ICT is clearly established, less is known about the economic burden of DFO treatment. Aim. To estimate the total annual costs of ICT from the National Health Service's perspective. Two generic HRQoL questionnaires, EuroQol (EQ-5D) and Short Form 36 (SF-36) have been used, higher score corresponding to better quality of life. Results. None of the 19 enrolled patients, aged 25-58 years (mean=33.2) developed inhibitors. Patients reported a mean of 2.37 events/patient/month during ODP (median=1.67, range: 0.5-15) vs. 0.48 events/patient/month during PP (median=0.25, range: 2.5) (Wilcoxon signed test p<0.001). Mean FVIII cost during ODP was 16,149 IU/patient/month during ODP (range: 2,000-50,000) and 26,342 IU/patient/month during PP (range: 16,667-38,333). Mean FVIII consumption was 16,149 IU/patient/month during ODP (range: 2,000-50,000) and 26,342 IU/patient/month during PP (range: 16,667-38,333). The mean cost to treat one bleed was 3,958 (median: 2,391). The incremental cost-effectiveness ratio, i.e. the cost for avoided bleed, was 4,675. At the end of the follow-up period, SF-36 showed a statistically significant improvement in patients quality of life in all domains (p<0.05). Concerning the Physical Component Summary score (PCS) and the Mental Component summary score (MCS), patients on PP showed better results than those on ODP, although no significant difference was found for MCS. Results obtained with EuroQol-5D were comparable to those shown by SF-36, with significantly different Visual Analogue Scale scores after ODP vs. after PP (p<0.05). Summary/Conclusions. These findings showed prophylaxis with RefactoTM dose of 25 IU/Kg tiw in adults with haemophilia was effective and safe. Our cost-effectiveness results can represent the point of reference for other similar evaluations. Furthermore prophylaxis has provided a significant improvement in HRQoL and it should therefore be considered in a cost utility evaluation.

0812 COST UTILITY ANALYSIS OF DEFERASIROX VERSUS DEFEROXAMINE FOR PATIENTS REQUIREING IRON CHELATION THERAPY IN THE UNITED KINGDOM
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Backgrounds. Patients suffering from β-thalassemia (β-thal), sickle cell disease (SCD), and myelodysplastic syndrome (MDS) require lifelong blood transfusions and are at risk of iron overload. If they are not treated with iron chelation therapy (ICT), they can suffer serious organ damage and reduced life expectancy. Desferal, infused subcutaneously for 8 hours per day, 5-7 times per week, is the standard of care for the depletion of excess bodily iron. Exjade is a new once daily oral iron chelation in UK treatment centers and the medical literature. Results indicated that patients with thalassemia, SCD, or MDS currently undergoing infusion ICT showed much lower HRQoL scores compared to population, and particularly for General Health, Role Physical, and Parent-Impact Emotional. Reducing the burden of treatment on the patient by having an effective, well tolerated, and more convenient therapy would contribute to improving the quality of life of these patients.
chelator, which has recently been approved by the FDA and Swissmedic for the treatment of transfusional iron overload. *Ann. To estimate the incremental cost per quality adjusted life year (QALY) of using Exjade instead of Desferal in patients with β-thal, SCD, or MDS who require iron chelation, from a UK NHS perspective.

### Table 1.

<table>
<thead>
<tr>
<th>Both case</th>
<th>Desferal</th>
<th>Exjade</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug costs</td>
<td>£ 3511</td>
<td>£ 11520</td>
<td>£ 7739</td>
</tr>
<tr>
<td>Administration costs</td>
<td>£7551</td>
<td>£6.0</td>
<td>£7545</td>
</tr>
<tr>
<td>Total costs</td>
<td>£ 11063</td>
<td>£ 11250</td>
<td>£ 187</td>
</tr>
<tr>
<td>Utility value</td>
<td>0.61</td>
<td>0.85</td>
<td>0.24</td>
</tr>
<tr>
<td>Incremental cost per QALY</td>
<td>£ 779</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Methods.

An aggregate annual cost of ICT with Desferal was informed by a primary study of 29 patients (11 β-thal; 14 SCD; 4 MDS; 31% male; mean age 30.6 ± 20.1 years, mean weight 54kg) from four UK treatment centers on the basis of chart reviews and interviews. Major resource items included drug costs, home delivery pumps and balloon infusors, and items relating to the use of portacaths. For Desferal, weighted mean prescribed annual dose frequency was 256, with a compliance rate of 83.7%; mean dosage was 37 mg/kg at a cost of £8.88/g. Exjade was assumed to have a prescribed frequency of 365 doses per year, with the same compliance rate as observed for Desferal (83.7%), and a dose of 20 mg/kg at a cost of £34/g. Unit costs (2004/2005 GBP) were applied. Costs related to monitoring were excluded, as were adverse events as a conservative assumption of equal compliance with Desferal and Exjade was defined. Annual utility values reflecting the impact of subcutaneous infusion compared with oral administration were estimated to be 0.61 and 0.85, respectively (Lawrence et al., ASH 2005). Results. In the base case analysis, Exjade has an extremely low incremental cost per QALY of £779, as shown in the Results, see Table. A range of one-way sensitivity analyses are also presented. *Conclusions. The once a day orally administered Exjade appears to offer an extremely cost-effective alternative to the current infusion-based iron chelator Desferal.

### COST-EFFECTIVENESS OF ALEMTUZUMAB IN PATIENTS WITH B-CELL CHRONIC LYMPHOCTIC LEUKEMIA (CLL) WHO HAVE FAILED ALKYLATING AGENTS AND FLUDARABINE

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**Backgrounds.** Currently, there are no cost-effectiveness data available comparing the use of alemtuzumab (MabCampath®) with alternative therapies for relapsed/refractory CLL after alkylating agents and fludara-bine, ie, third-line patients. AIM: The objective of this analysis was to estimate the cost-effectiveness of 8 weeks of alemtuzumab administered intravenously (IV) or subcutaneously (SC) when compared with 1) an alternative antibody; high-dose rituximab, 2) 6 cycles of CHOP; or 3) continuation of investigator's standard therapy. Methods. The effectiveness evidence used for analysis was derived from a review of published clinical studies enrolling patients who failed fludarabine, except in the case of CHOP, where the limited data available were mostly based on patients with less advanced disease. *Expected months in remission per patient treated* was used to assess the health benefit of each therapy, and was estimated by multiplying the weighted overall response rate by the duration of response. In addition, for comparison between alemtuzumab and CHOP, life-months gained were calculated. Resource use associated with each intervention was based on the published literature, a previous patient level costing study conducted in the Netherlands in follicular lymphoma, and expert opinion. Utilisation costs for the Netherlands were derived from local hospital accounting systems, published tariffs, and listed wholesale prices. Sensitivity analysis was used to test the robustness of the findings. Unit costs were based on the 2003 price year. Results. Mean total cost of treatment with alemtuzumab IV was calculated to be approximately €25,281. Savings associated with a switch from IV to SC alemtuzumab were €5,053 with hematologist administration and higher with self-administration. Although cost of treatment with CHOP, £7,174, is lower than with alemtuzumab, in terms of health benefit the expected number of months in remission per patient treated is 3.61 months with alem- tuzumab versus 1.59 months for CHOP. Cost of 12-dose rituximab, €30,155, is higher than alemtuzumab, and the expected time in remis- sion is less at 1.98 months. Comparison of health benefits for each ther- apy with their cost shows that the mean costs per month in remission for alemtuzumab and CHOP are within a similar range: CHOP is €4,519 as base-case scenario, alemtuzumab SC 5,608, and alemtuzumab IV €6,449. For 12-dose rituximab the cost per month in remission is consi- derably higher at €15,195. When compared with a historical chemotherapy control, alemtuzumab is associated with a survival gain of approximately 8 months. Assuming this level of health benefit over CHOP would lead to an incremental cost per life-year gained for alem- tuzumab IV of €24,160. *Conclusions. This analysis shows that for each third-line IV chemotherapy treatment with alemtuzumab IV is used instead of CHOP, additional cost to the payer would be on average €16,107, or less at 13,072 for SC administration by a hematologist. Benefit to the patient would be 8 months of survival gain, on average. The associated incre- mental cost per life-year gained for alemtuzumab IV over CHOP would be €24,160, well within the accepted range. Furthermore, when the costs and benefits of alemtuzumab are compared with high-dose ritux- imab monotherapy, alemtuzumab is both less expensive and more effective.

### 0814

**A QUALITY MANAGEMENT SYSTEM FOR JACIE ACCREDITATION AT MINIMAL FINANCIAL COST**

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**Backgrounds.** The Joint Accreditation Committee of the International Society for Cellular Therapy and the European Blood and Marrow Transplantation Group (JACIE) offers an accreditation programme to trans- plants centres on the basis of chart reviews and interviews. Major resource items included drug costs, home delivery pumps and balloon infusors, and items relating to the use of portacaths. For Desferal, weighted mean prescribed annual dose frequency was 256, with a compliance rate of 83.7%; mean dosage was 37 mg/kg at a cost of £8.88/g. Exjade was assumed to have a prescribed frequency of 365 doses per year, with the same compliance rate as observed for Desferal (83.7%), and a dose of 20 mg/kg at a cost of £34/g. Unit costs (2004/2005 GBP) were applied. Costs related to monitoring were excluded, as were adverse events as a conservative assumption of equal compliance with Desferal and Exjade was defined. Annual utility values reflecting the impact of subcutaneous infusion compared with oral administration were estimated to be 0.61 and 0.85, respectively (Lawrence et al., ASH 2005). Results. In the base case analysis, Exjade has an extremely low incremental cost per QALY of £779, as shown in the Results, see Table. A range of one-way sensitivity analyses are also presented. *Conclusions. The once a day orally administered Exjade appears to offer an extremely cost-effective alternative to the current infusion-based iron chelator Desferal.*
hhood that an event with relevance to quality enhancement will pass unreported. The software is free of charge, and installation takes around 15 hours of paid collaborator time. The solution can be shared on request, conform GPL. It is extendable for documenting educational JACIE needs. Screenshots will be presented. Results and conclusion A tool for event reporting was established at minimal cost through open source software. It guarantees indefinite availability of data and anticipates future legislation. It contributes to a quality management system as needed for JACIE accreditation.

**Allogeneic stem cell transplantation II**

**0816 IMMUNOLOGIC RECOVERY AND GRAFT VERSUS HOST DISEASE AFTER NONMYELOABLATIVE STEM CELL TRANSPLANTATIONS**

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**Background.** Non myeloablative stem cell transplant (NSCT) is an important therapeutic option for those patients (pts) who are not eligible to conventional transplant The curative potential of these transplant plants are directly related to the anti tumor effect of the graft and rapidity of donor T cells engulfment. Methods. We have thus investigated the cellular immunologic recovery in 80 pts (20-64 years old) given peripheral blood stem cells from Matched Related and Unrelated Donors, mostly receiving a conditioning regimen based on fludarabine, Antithymocyte globulin (ATG) and Cyclophosphamid (or cytarabine or melphalan). Post grafting immunosuppression usually consisted of cyclosporin and mycophenolate mofetil. The mononuclear blood cell subsets were assessed by flow cytometry; analyses were performed on days 80, 160, 180, and 360 after SCT. Incidence of graft versus host disease (GVHD) and opportunistic infections was correlated with immune recovery. Results. We observed an early recovery of CD8 T cell (d8) and NK cells (d22) whereas CD4 T cells remain below the normal value until d360. This slow CD4 T cell recovery was not correlated with the total T cell number in graft in our series. The GVHD rate was similar to classical SCT -maybe with a lower severity- but mostly delayed compared to classical SCT. Our data show that a low rate of CD4 T cells does not protect from GVHD, but this delay in T cell recovery might explain the late occurrence of GVHD in NSCT and also a later CMV-specific immune reconstitution translated into an increased frequency of CMV-reactivation. However, this did not lead to increased CMV diseases. We also observed a higher rate of CMV infections when CD4 T cells were below 100/µL. Invasive fungal infections were not correlated with CD4 T cells recovery in our study and mostly observed in patients receiving steroids for GVHD. 80% of patients in complete response after NSCT had developed a GVHD. Donor lymphocyte infusion was mostly useful to salvage relapsing who failed to present a GVHD after SCT. Conclusions. Our small series confirms the good tolerance of these NSCT, a late and low rate occurrence of GVHD using ATG in the conditioning regimen, and an increased rate of CMV infections correlated with a low count of CD4 T cells.

**0817 LOW-DOSE METHOTREXATE AS SALVAGE THERAPY FOR REFRACTORY GRAFT-VERSUS-HOST DISEASE AFTER REDUCED-INTENSITY CONDITIONING FOR ALLOGENEIC STEM CELL TRANSPLANTATION**

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Institut Paoli-Calmettes, Marseille, France

Corticosteroid (CS)-resistant GVHD is associated with high morbidity and mortality, and therapeutic options are limited for those patients. Also, elderly patients undergoing RIC allo-SCT are more exposed to the side effects of long term CS administration. Low dose methotrexate (MTX) therapy is a well established modality for GVHD prophylaxis. However, little is known about the role of this drug in CS-resistant GVHD. This pilot single center study investigated the role of MTX in a curative setting after failure of CS treatment in patients undergoing RIC allo-SCT. 20 patients received IV infusions of low dose MTX (5 mg/m²/infusion) at weekly intervals, for at least 4 weeks. Reasons for MTX administration were: CS-refractory acute GVHD, CS-refractory chronic GVHD, chronic GVHD exacerbation after CS taper, or CS severe side effects. Responses to low dose MTX infusions were assessed one month after the last infusion in each involved organ. 12 patients were treated for severe acute GVHD, while 8 patients received MTX for extensive chronic GVHD. Median age of patients was 51 (range, 22-60). Median time of administration of MTX was day +89 (range, 30-300). Of note, none of the patients received any other concomitant therapy for refractory GVHD. 13 patients responded to MTX administration (65%) with 5 complete responses (25%). Among the 12 patients treated for acute GVHD, 7 responded (58%) of whom 5 CRs (42%). 8 patients did not respond and died from resistant GVHD. Interestingly, 5 patients...
from the group of grade 3-4 acute GVHD responded. Among the 8 patients treated for chronic GVHD, 6 were responders (75%). In addition, MTX allowed a significant reduction of CS daily dosage ranging from 25% to 80%, as assessed one month after the last administration of MTX. With a median follow-up of 287 days, no increase of CS therapy was necessary among these 6 MTX-responder patients. In all, toxicity of low dose MTX administration was low (transient and mild reversible cytopenia in 5 cases, 15%). Among the 20 patients, 14 are still alive (70%) with a median follow-up of 293 days (range, 65-515) days. Overall, 2 patients died of progressive disease, while 4 patients died from refractory GVHD. We conclude that low dose MTX is a well-tolerated, inexpensive and likely steroid-sparing agent that is worthy of further investigation in prospective trials for treatment of refractory GVHD, but also as frontline therapy in combination with CS.

0818

OBSERVATION-BASED EARLY WARNING SCORES TO DETECT IMPENDING CRITICAL ILLNESS PREDICT IN-HOSPITAL AND OVERALL SURVIVAL IN PATIENTS UNDERGOING ALLOGENIC STEM CELL TRANSPLANTATION

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Backgrounds. Observation-based early warning scoring systems have been developed to improve the outcome of critically ill patients by triggering early critical care intervention. To date none of these scoring systems have been validated in cancer patients or stem-cell transplant recipients. Aims. The aim of this study was to validate three established scoring systems in adult recipients of Allogeneic stem cell transplantation (Allo-SCT) and to determine their usefulness at predicting survival. Methods. We retrospectively analysed the physiological observations during the initial admission of patients undergoing Allo-SCT. Three different early warning scoring systems termed MEWS, PARS and LEWS (Table 1) were assessed.

Table 1. Leeds based modified early warning score (LEWS).

<table>
<thead>
<tr>
<th>Score</th>
<th>Heart Rate (beats/min)</th>
<th>Systolic blood pressure (mmHg)</th>
<th>Respiratory rate (min-1)</th>
<th>Oxygen saturation (%)</th>
<th>Respiratory support</th>
<th>Urine output in last 4 hours</th>
<th>Level of consciousness</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>&lt;40</td>
<td>70-80</td>
<td>&lt;8</td>
<td>85-89</td>
<td>BIPAP/CPAP</td>
<td>&lt;80</td>
<td>Confusion</td>
</tr>
<tr>
<td>2</td>
<td>41-50</td>
<td>81-100</td>
<td>8-11</td>
<td>90-94</td>
<td>H/Flow Oxygen Therapy</td>
<td>80-120</td>
<td>Alert</td>
</tr>
<tr>
<td>1</td>
<td>51-100</td>
<td>101-179</td>
<td>12-20</td>
<td>90-94</td>
<td></td>
<td>120</td>
<td>Reacts to voice</td>
</tr>
<tr>
<td>0</td>
<td>101-110</td>
<td>180-199</td>
<td>21-25</td>
<td>&gt;95</td>
<td></td>
<td>200</td>
<td>Reacts to pain</td>
</tr>
<tr>
<td>1</td>
<td>111-130</td>
<td>200-220</td>
<td>26-30</td>
<td></td>
<td></td>
<td>799</td>
<td>Unresponsive</td>
</tr>
<tr>
<td>2</td>
<td>&gt;130</td>
<td></td>
<td>&gt;30</td>
<td></td>
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</tr>
</tbody>
</table>

Results. Charts of 43 patients (AL n=21, HD/NHL n=10, MM n=4, CML n=7, SAA n=1) with a median age of 40 years (IQR 29-49) were analysed. 29 of 43 patients received grafts from matched sibling donors and 18 of 43 received radiation-based full intensity conditioning. Impairment of respiratory function was the commonest (40 patients, 93%) event, usually deteriorating during the second week post-graft. All scores revealed high accuracy in predicting in-hospital survival (AUC in ROC for MEWS 0.915, for PARS 0.985 and for LEWS 0.988 respectively, p < 0.001). For all three scores the cut-off level associated with a high risk of event, usually deteriorating during the second week post-graft. All scores revealed high accuracy in predicting in-hospital survival (AUC in ROC for MEWS 0.915, for PARS 0.985 and for LEWS 0.988 respectively, p < 0.001). For all three scores the cut-off level associated with a high risk of event, usually deteriorating during the second week post-graft.

0819

A DOSE FINDING STUDY OF IV BUSULFAN IN COMBINATION WITH CYCLOPHOSPHAMIDE AS CONDITIONING REGIMEN PRIOR ALLOGENIC STEM CELL TRANSPLANTATION IN CHILDREN WITH MALIGNANT AND NON-MALIGNANT HAEMATOLOGICAL DISEASES: OPTIMISATION OF BUSULFAN TREATMENT

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1Hopital Saint Louis, PARIS, France; 2Institut Gustave Roussy, VILLEJUIF, France; 3Hopital Debrousse, Lyon, France; 4Hopital Hôtel Dieu, NANTES, France; 5Hopital la Timone, MARSEILLE, France; 6Hopital Necker-Enfants Malades, PARIS, France; 7Hopital Robert Debére, PARIS, France; 8Hopital d’Enfants Brabois, VANDOEUVRE LES NANCY, France; 9Institut de Recherche Pierre Fabre, BOULOGNE BILLANCOURT, France

Backgrounds. In pediatric patients (pts) oral busulfan (Bu) is often included in conditioning regimens prior to allogeneic (allo)-haematopoietic stem cell transplantation (HSCT). Bu has a narrow therapeutic window and under- or overdosing may have a fatal outcome. Bu clearance (Cl) is high in children and higher doses are needed to obtain an area under the curve (AUC) equivalent to adults. To optimise Bu treatment we defined (Nguyen L. et al BMT 2004) and assessed prospectively a new IVBu fixed dosing allowing to 91% of 55 pts targeting AUC (900-1500 µM.min) without therapeutic drug monitoring (TDM) (Pharmacokinetic results were reported separately by G Vassal et al). We report here the clinical outcome of these children after allo-transplant. Aims. To investigate the safety of this new IVBu dosing strategy, to assess whether these doses are myeloablative and supportive for engraftment, and to evaluate the consequences of IVBu dosage upon children clinical outcome Methods. Children (15 male/13 female) received IVBu-based Bu-cyclophosphamide (Cy, 200mg/kg) prior to HSCT. IVBu (over 2 h infusion) was given based on body weight: 1.0 mg/kg, 1.2 mg/kg, 1.1 mg/kg, 0.35 mg/kg, and 0.8 mg/kg for pts with <9 kg, 9 to <16 kg, 16-23 kg, >23-34 kg, and >34 kg strata of weight, respectively. Clonazepam was given as seizures prophylaxis. Indications for HSCT were: AML (n = 14, 12 CR1, 1 CR2, 1 FR), CMl (n = 8), ALL (n = 1), MDS (n = 1); hemoglobinopa thy (n = 6), and immunodeficiency (n = 5). Recipients aged from 0.3 to 17.2 years (median 7.2 y) received bone marrow containing 5.7 × 10^8/kg and >34 kg strata of weight, respectively.

Conclusions. In this study we report the clinical outcome of the children treated with IVBu. The majority of patients had a full engraftment (63/77) with G I-II 27 pts, G III 25 pts, and G IV 19 pts. There were no severe haematological toxicities, but 4 pts (5%) died due to severe GVHD (n = 1), AML relapse (n = 2), and poor neurological outcome (n = 1). Overall, 22 pts (29%) were in CR at discharge, and 14 pts (18%) are in CR at last follow-up. The most likely time to develop clinical deterioration is the second week post-graft infusion.
Daclizumab, a humanised monoclonal antibody against interleukin-2 receptor, has been used in steroid-refractory acute graft-versus-host disease (aGVHD). Reported results were conflicting. Aims and Methods. We performed a retrospective audit of the outcome data of 12 consecutive allograft patients who had been treated with Daclizumab for steroid-refractory (to > 2mg/kg/d of iv Methylprednisolone) grade III-IV aGVHD from year 2000-2004 in our unit. All patients received standard anti-microbial prophylaxis, and Cyclosporin and Methotrexate GVHD prophylaxis, except for three reduced-intensity allografts who received cyclosporine alone. Clinical grading of aGVHD was performed according to standard criteria. 1mg/kg of iv Daclizumab was given on days 1, 4, 8, 15 and 22 and definition of treatment response as previously described (Przepiorka 2000). Results. Twelve patients developed grade III-IV aGVHD after HLA-matched blood stem cell allogeneic transplants, which consisted of 9 sibling (7 ablative, 2 reduced-intensity) and 3 unrelated (1 ablative, 2 reduced-intensity) allografts. Daclizumab was commenced after failure of iv Methylprednisolone at a median of 81/2 days (range: 3-28). These patients also received numerous (range: 8-7) concomitant GVHD therapies, including Steroids, Cyclosporin/Tacrolimus, Mycophenolate, and Etanercept (for gut aGVHD). Anti-thymocyte globulin (ATG) was additionally given for poor responders to Daclizumab in 6/12 patients. The only complete responder to Daclizumab (patient 7) died of subsequent exacerbation of gut GVHD, haemorrhagic cystitis and CMV viraemia. The single partial responder (patient 6) eventually died of progressive gut GVHD and bacterial sepsis. There was no long-term survivor with infections as terminal events in 10/12 patients. Conclusions. In contrast to initial published reports, allograft patients with severe steroid-refractory aGVHD had poor response and dismal outcome when treated with Daclizumab in our institution. It was our major concern that the poor survival may be contributed by the delay of more appropriate GVHD therapy and the aggravation of infective complications. As a result, we have moved away from Daclizumab back to ATG since 2005. Novel GVHD therapies such as photopheresis, mesenchymal stem cells should be explored.

Table 1. Pre- and post-Daclizumab responses and outcome.

<table>
<thead>
<tr>
<th>Score</th>
<th>Skin GVHD</th>
<th>Gut GVHD</th>
<th>Liver GVHD</th>
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<td>Death, d60</td>
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<td>3</td>
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<td>3</td>
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<td>0</td>
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<td>0</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>Death, d31</td>
</tr>
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<td>6</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>Death, d345</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
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<td>Death, d232</td>
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<td>8</td>
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<td>12</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>Death, d166</td>
</tr>
</tbody>
</table>

0821

ALLOGENEIC STEM CELL TRANSPLANTATIONS AFTER REDUCED INTENSITY CONDITIONING REGIMEN FLUDARABIN, BUSULFAN AND ATG (FRESENIUS)

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Introduction: Allogeneic stem cell transplantation with reduced intensity conditioning (RIC) is effective therapy for hematological diseases. Methods. This is a retrospective report about 63 patients [21 women, 42 men, median age 51 years (15-65)] who underwent hematopoietic stem cell transplantation (HSCT) after reduced intensity conditioning regi-

men (RIC) with Fludarabin (30mg/m2, 5 days), Busulfan (8-12 mg/kg p.o.) and ATG Fresenius (10mg/kg/d, 4 days) for hematological disease in our transplant center between March 1998 and December 2005. The diagnosis were 17 AML [15 in 1st.CR, 1 in 2nd CR, 1 in relaps (R)], 3 MDS, 1 AA, 22 CML (21 in chronic phase, 1 in acceleration), 1 myelofibrosis, 3 HD (1R, 1 CR, 1 PR), 8 B-NHL (2 DLBCL, 1 FL, 3 MCL, 2 SCLL) (1R, 4 FR, 3 CR), 6 CLL (4 R, 2 FR), 2 MM (1R, 1 FR). Peripheral blood stem cells (PBSC) were used in 60 patients, bone marrow in 3 patients. Median of infused CD34+ cells was 6.76×10^6/kg. Donors were 57 registred and 6 unrelated respectively. As GVHD prophylaxis, 58 patients received CsA, 2 received CsA+MTX and 3 CsA+ mycophenolate mofetil. Results. Median time of follow up was 25 months. After transplantation, any toxicity was observed in 25% (16) patients, 66% (42) patients developed a toxicity grade III, 7% (9) patients a toxicity grade II, 2% (1) patient a toxicity grade IV.

Without any parenteral nutrition were 68% (43) patients, and 32% (20) patients have parenteral nutrition in median 11 days (3-22 days). Recovery of neutrophils(>1.0×10^9/L) was in median time 18 days, trombocytes (>20×10^9/L) in median time 13 days. Complete chimerism (CC) was reached in median time 73 days in 49 (78%) patients, 2 (3%) patient still didn't reach CC. CC 2 (5%) patients didn't reach CC for short time after transplantation, the others didn't reach CC because of rejection of graft (1), giving his autologous back up of stem cells for severe GVHD (1), relaps of disease (4pts=7%), death from other reason (5 infections, 1 bleeding). Twenty-two (35%) patients developed an acute GVHD: 9 patients maximal grade I, 8 patients maximal grade II, 5 patients maximal grade III, 2 patients grade IV. A chronic GVHD was presented in 28 (44%) patients (23 limited, 5 extensive). Secondary rejection of graft occurred in 2 patients with unrelated donor. Fourteen patients had preemptive therapy of CMV. Any infection since day +100 had 28 (44%) pts, after day +100 51 (49%) patients. Twenty patients (31%) died, the causes of death: 10 (15%) relapses of disease, 3 (5%) infections, 4 (6%) GVHD, 1(2%) toxicity, 2 (3%) from other reason. Early transplant related mortality (TRM) was 10% (6 patients: 2 relaps, 1 GVHD, 1 bleeding, 2 infections), late TRM 5% (8 patients GVHD). Median time of overall survival for all patients (Kaplan-Meier) wasn't reached, AML patients had 46 months, CML patients 6-4 months and CML patients and B-NHL patients wasn't reached. Conclusions. RIC is associated with favorable outcome and low toxicity in patients in remission at the time of transplantation.

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LOW-DOSE METHOTREXATE AS SALVAGE THERAPY FOR REFRACTORY GRAFT-VERSUS-HOST DISEASE AFTER REDUCED-INTENSITY CONDITIONING ALLOGENEIC STEM CELL TRANSPLANTATION

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Corticosteroid (CS)-resistant GVHD is associated with high morbidity and mortality, and therapeutic options are limited for those patients. Also, elderly patients undergoing RIC allo-SCT are more exposed to the
side effects of long term CS administration. Low dose methotrexate (MTX) therapy is a well established modality for GVHD prophylaxis. However, little is known about the role of this drug in CS-resistant GVHD. This pilot single center study investigated the role of MTX in a curative setting after failure of CS treatment in patients undergoing RIC allo-SCT. 20 patients received IV infusions of low dose MTX (5 mg/m²/day) during 28 days. Follow up ranges from 273-2068 days. Results. Median age for BM and PBSC group was 5.4 and 6.2 years. Engraftment was achieved in all of CS. Median time for ANC of ≥0.5x10⁶/L in BM/PBSC patients was 15/10 days (range 11-19.9/15) and for platelets of 20x10⁹/L it was 17/14 days (range 14/28/12-19). aGVHD (grade II-IV) was seen in 30%/26% cases in BM/PBSC group. Incidence and severity of chronic GVHD was not statistically different in two groups (BM-24%/PBSC-30%). Six patients rejected the graft: 2 in BM group and 4 in PBSC group. Of the four who rejected the graft from class III, 3 are from PBSC group. DFS in risk classes of the two groups is not significant. Overall survival/disease free survival for the BM and PBSC group as on December 2005 is 73%/65% and 67%/55%. Conclusion: The results of PBSC as a whole or according to risk class remains comparable to BMT. A trend towards less rejection and better disease free survival is seen in class III patients who received BM harvest, but this is not statistically significant.

0824 ALLOGENIC STEM CELL TRANSPLANTATION 1985-2005. A SINGLE CENTER EXPERIENCE

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Backgrounds. From 1985 to 2004, 398 adult patients underwent allogeneic stem cell transplantation (ASCT) in our institution. In order to perform a quality control and compare our results with those of other centers, we evaluated the overall survival and the incidence of complications following ASCT. Design and Methods. 398 patients received ASCT for hematological malignancies or severe aplastic anemia (SAA); 42.5% female (N=169) and 57.5% (N=229) male. Median age was 39.0 years; during the study period the median age increased significantly (p=0.001). 273 patients (68.8%) received transplants from family donors and 124 patients (31.2%) from matched unrelated donors (MUD). On patient died during conditioning prior to transplantation. Eligible donors included siblings sharing both HLA haplotypes and haploidentical family donors with a maximum of one antigen mismatch on A or B (serology) or DRB1 on the non-shared haplotype, and MUD identical on A and B (serology), and received G-CSF 5mg / kg / day, from day +5, till ANC 200 started on day-10. Triple immunosuppression was used for PBSC: Hydrea 20-30 mg/kg (day - 45 to -11), Azathioprin 3 mg/kg (day - 45 to -11), CyA 1-2 mg/kg (day - 45 to -11) and received desferioxamine, 24 hours infusion, before transplantation. Most patients were intensely transfused to keep haemoglobin 13-14 gm/dl and received desferioxamine, 24 hours infusion, before transplantation. Overall survival (OS) was 58%; disease free survival (DFS) 53%; 5 year OS was 58%; 5 year DFS was 53%. Cumulative incidence of acute GvHD grade II-IV was 32% and 37% in BM and PBSC group as on December 2005 is 73%/65% and 67%/55%.

0825 EXTRACORPOREAL RADIOPHERESIS FOR ACUTE AND CHRONIC GRAFT VERSUS HOST DISEASE

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Graft vs host disease (GVHD) is the main immunological complication of hematopoietic transplants. Unfortunately pharmacological therapies do not always effectively control severe and progressive cases. Extracorporeal photopheresis has been proposed as an effective procedure to control GVHD treatment. As an alternative to this treatment, we evaluated the action Extracorporeal radiopheresis (ex vivo leukocytes irradiation with minimal doses of γ irradiation). Clinical, immunological and skin histological evaluation of patients with acute and chronic GVHD who received only pharmacological therapy were compared with
patients who received the pharmacological therapy plus Extracorporeal
radiopheresis was evaluated. 15 patients with aGVHD and 15 with cGVHD were studied. In aGVHD
grade IV neither treatment affected the long-term survival. Nevertheless, in 3/4 patients (75%) who received radiopheresis, diminution of clinical
symptoms (gastrointestinal bleeding and skin rash) was observed since the 1st week of therapy, improving the quality of life. This
effect was also observed at later in patients receiving only pharmacological
therapy. In cGVHD, 7 patients received radiopheresis, 5 (71.4%),
proved the skin pain since the first week and the skin sclerosis after 6-12 months. In one patient, control of cGVHD progression was obtained.
20/24 patients had previously received pharmacological therapy without control of the GVHD. 1/8 patients that received only pharmacological
therapy, three improved (37.5%), in three (37.5%), were control of cGVHD progression. Two got worse (25%). Histological
skin follow-up showed that in aGVHD severity score were one grade lower on all radiopheresis cases evaluated. In patients that received
pharmacological therapy only, 50% were the same grade, 20% got worse. For cGVHD skin biopsies were made after 6-12 months. Lower
or same histological grade were observed in six patients that received radiopheresis and four patients with only pharmacological therapy, 87.5% and 50% respectively. Induction of apoptosis in cells that received radiopheresis was evaluated with DIOC-6/BD. No changes in the phenotype of dendritic cells differentiated from monocytes with IL-4+GM-CSF were observed. Summary: Better clinical and histological therapeutic
effects were observed on patients who received Extracorporeal Radiopheresis. Multicentric studies will contribute to evaluate this therapy in a larger number of patients.

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IMMUNOHISTOCHEMICAL EVALUATION OF INfiltrATING CELLS
(CD4, CD8, AND CD56) AND LANGERHANS CELLS IN SKIN OF GRAFT VERSUS HOST
Disease Patients
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Graft vs. host disease (GVHD) occurs in about 60-80% of allogeneic
hematopoietic stem cell transplant recipients due to mismatching of major and minor histocompatibility antigens. Cutaneous involvement is
a frequent clinical manifestation of GVHD. In this regard, Langerhans cells (LC) have been shown to play an important role in the pathogenesis of GVHD as antigen-presenting cells through both the direct and indirect allo-recognition pathways. Thus, we carried out an immunohistochemical study to determine the characteristics of the infiltrating T cells (CD4+ and CD8+) and NK cells (CD56+) as well as LC (CD1a+) on skin biopsies of GVHD patients and their correlation with global severity scores. Forty-two patients were allo-transplanted between June, 1998 and December, 2002. Twenty-nine (69%) patients
developed GVHD; among these, 15 (36%) developed acute GVHD, 5 (11.9%) developed chronic GVHD, and 9 (21.4%) developed acute and chronic GVHD. Immunohistochemical enumeration of CD1a+, CD4+, CD8+, and CD56+ cells were performed in paraffin-embedded punch skin biopsies taken mainly from the thorax. Among the 24 cases with acute GVHD, skin involvement was observed in 23/24 (95.8%) patients, most of them with G-I-II scores. Intestinal GVHD was observed in 20/24 (83.5%) patients with 15 (75%) patients with G-I-II scores and 5 patients (25%) with G-III-IV scores. Hepatic GVHD was observed in 8 (33.3%) patients, 5 (62.5%) of those patients with G-I-II scores. The number of LCs/mm2 in dermis and epidermis was significantly lower in cases with major global severity scores: Normal skin donors: (mean±SD) 15.6±1.6, acute GVHD G-I-II: 7.5 ± 8.8 and G-III-IV: 3.6±2.7 (p<0.05). An increase in the ratio of infiltrating perivascular T cells to LC was observed and it was inversely proportional to the number of LCs on epidermal and dermal layers of the skin. There was no increase of CD56v NK cells in patients with acute GVHD as compared to normal controls. Figure 1. Extensive chronic GVHD was seen in 7/14 (50%) patients. The number of LC was similar in limited and extensive chronic GVHD (9.5±4.2 and 9±12.7, respectively). In de novo chronic GVHD, the number of LCs was higher (15±4.4), than in progressive (7.3±0) or quiescent (2.7 ±
3.9) GVHD (p=0.05). Scleroderma-like presentation showed higher number of LC (9.7±6.7) as compared to lichen-like lesions (7.3±0).

No increase in the ratios of infiltrating epidermal and perivascular CD8+, CD4+ or CD56+ cells was observed. In summary, in acute severe
systemic GVHD, a significantly lower number of LCs and higher number of CD8+ T cells were observed. These changes were not observed in chronic GVHD. This study indicates that skin CD8+ T cell/LCs ratios could be used as an additional tool for diagnosis and follow-up of GVHD.

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**ALLOGENEIC BONE MARROW TRANSPLANTATION IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA**


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Background and Aims. Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired chronic clonal hematological disorder of pluripotent stem cells. That disease may be complicated by myelodysplastic syndrome and leukemic conversion. Currently, allogeneic bone marrow transplantation (BMT) is the only curative approach. Patients and Methods. Between November 1990 and October 1998, six patients (2 F/4 M) with a median age of 26.5 years (range 17-38) underwent BMT for PNH at our institution. BMT was done due to severe progressive cytopenias in five patients and frequent recurrent hemolytic crisis in one. Median time from diagnosis to BMT was 40 months (range 7-58). Five patients were transfusion-dependent and three of them had received several lines of therapy before BMT (steroids, immunsupresors and danazol). Four donors were genotypic HLA-identical siblings, one was a non identical sibling donor (major mismatch to one class A HLA antigen) and the remaining one was an unrelated HLA-identical donor. Conditioning regimen consisted of busulphan (40 mg/kg) and cyclophosphamide (150 mg/kg). Graft versus host disease (GVHD) prophylaxis consisted of cyclosporine and a short course of methotrexate. Results. The median number of nucleated cells infused was 2.1×10^9/kg (range 1.8-3.9). Time to achieve a granulocyte count > 0.5×10^9/L and a platelet count > 50×10^9/L was 17 and 82 days, respectively. Full donor chimeras was observed in every case, although one patient presented mixed chimeras 9 years after BMT. In this case, peripheral blood stem cells (PBSC) from the same donor were infused without previous conditioning. Currently, five months after this PBSC infusion the patient is well with complete chimaera but with thrombocytopения. Overall, four patients developed acute GVHD (one grade I and 3 grade III-IV). Chronic GVHD was extensive in four patients. Four patients are alive at +102, +118, +142 and +182 months, and two patients died at 11 and 97 months after BMT because of septic shock. Conclusions. Allogeneic BMT is a curative and suitable approach for selected patients with severe PNH. BUCY2 as conditioning regimen was able to eradicate the abnormal PNH clone. GVHD is the complication most frequently observed.

**ALLOGENEIC STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA**


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Backgrounds. HD chemo/radiotherapy followed by autologous stem cell transplantation (SCT) has been associated with improved outcome in MM. Unfortunately, following autologous SCT almost all pts had progressive disease. Aims. We evaluated the outcome of 29 pts (17 M, 12 F) with stage III MM treated with hematopoietic SCT. Twenty-three, 6 and 1 pts underwent a matched sibling donor allogeneic transplant after a reduced intensity conditioning regimens (RIC), a matched sibling donor SCT and an unrelated hematopoietic SCT, respectively. Twenty-two pts were treated with autografting followed by reduced intensity conditioning allotransplantation. All these patients received HD Melphalan (200 mg/m^2) followed by autologous PB-SCT. After a median of 90 days, the pts underwent RICT (Fludarabine + 2 Gy TBI). Acute GVHD prophylaxis consisted of MM and cyclosporine. Chimerism analysis was performed using STR-PCR and donor engraftment was evaluated at day +15,+30,+45,+60,+90 on unfractonated BM cells. All pts received a HLA identical donor mobilized PBSC and the graft contained a median of 3,8×10^5 (range 1.6-8) *10^6 cells/kg body weight. After RICT, on day +15, 3 (13%) pts showed a complete donor chimerism; on day +90, 21 (90%) showed a complete donor chimerism; two pts with mixed chimerism received a DLI on day +30 and one of these achieved full donor chimerism. Results. Grade II-III acute GVHD occurred in 4 pts (17%) but no patient died. Five patients (22%) developed a mild and 6 (26%) an extensive chronic GVHD . After RICT B pts (35%) achieved CR and they are in CCR at +57,+57,+51,+49,+46,+20,+14 +15 months; 2 (9%) pts show near CR and 3 (13%) are in PR. Ten pts not in CR showed a progressive disease and six of these died. With a median follow-up of 22 months, 17 (74%) are alive. Six and one patients received a related and unrelated hematopoietic SCT, respectively. The pre-transplant high-dose preparative regimen included CY-TBI. On day 0 all collected PBSCs were infused. GVHD prophylaxis included cyclosporine and short-course methotrexate; ATG was added in the unrelated transplant. All patients showed a complete donor chimerism at the time of engraftment. Grade II-IV acute GVHD occurred in 2 pts and 1 of these died. All pts developed mild chronic GVHD. One patient relapsed and died 24 months after allogeneic SCT. To date, 5 patients are alive and 3 of them are in CCR at +28 +4 (ALLO) and +21 (MUD) months. Conclusions. We demonstrated that survival after allogeneic transplantation is favourable: 74% of all pts achieved CR or PR. The 100-day TRM was low (only 4%) and no patient died after RICT. Pancycopenia after RICT was minimal and sustained allogeneic stem cell engraftment occurred in 95% of patients. A good correlation between GVHD, full chimerism and remisison was found. All patients in CR or NCR developed acute/chronic GVHD and the presence of GVHD correlated with a lower relapse rate. In all patients (RICT, ALLO TMO and MUD) the achievement of CR was gradual and a constant regression of the monoclonal band was observed.
TACROLIMUS AND METHOTREXATE FOR THE PROPHYLAXIS OF GRAFT-VERSUS-HOST DISEASE AFTER UNRELATED DONOR CORD BLOOD TRANSPLANTATION FOR ADULT PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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Backgrounds. Although allogeneic cord blood transplantation (CBT) has been increasingly used as a therapeutic option for hematologic malignancies, the prophylaxis of GVHD has varied significantly among different studies and has included cyclosporine A (CSA) alone or in combination with prednisolone/methylprednisolone, short-term methotrexate, or anti-thymocyte globulin. Aims. We present the outcome of CBT for adult patients who received tacrolimus and short-term methotrexate (MTX) for GVHD prophylaxis. Patients and Methods. Eighteen patients with hematologic malignancies underwent cord blood transplantation (CBT) from unrelated donors after having been conditioned with myeloablative (n=13) or reduced-intensity (n=5) regimens and received tacrolimus and methotrexate (15 mg/m² on days 1, 10 mg/m² on days 3 and 6) as a graft-versus-host disease (GVHD) prophylaxis. The median number of nucleated cells of infused cord blood was 2.66×10¹⁰/kg of patient body weight. Results. Engraftment was achieved in 16 of the 18 patients. The median time to absolute neutrophil count >0.5×10⁹/L was 21.5 days (range 17-32), and the median time to platelet count >2.0×10⁹/L was 36 days (range 26-57). Of the 16 evaluable patients, 5 and 8 patients had grades I and II acute GVHD, respectively, and none had grades III/IV acute GVHD. The cumulative incidence of grade II acute GVHD was 44.4%. Chronic GVHD occurred in 7 of 15 evaluable patients (limited-type 3, extensive-type 4). Infections complications were common, including septicemia in 10 patients, CMV disease in 3 patients, and fatal invasive aspergillosis in 1 patient. Of the 18 patients, 14 were alive and disease-free between 173 and 1514 days after CBT (median 746 days), and the probability of disease-free survival at 2 years was 79.1%. Conclusions. Our results suggest that tacrolimus and short-term methotrexate effectively prevent the occurrence of severe acute GVHD after unrelated CBT, and could contribute to a higher survival rate, although the management of infectious complications is essential.

INCREASING MIXED CHIMERISM DETECTED WITH SHORT TANDEM REPEATS DEFINES A GROUP OF PATIENTS WITH POOR OUTCOME AFTER REDUCED-INTENSITY CONDITIONING ALLOGENEIC PERIPHERAL BLOOD STEM-CELL TRANSPLANTATION WHICH CAN BE IMPROVED BY IMMUNOTHERAPY

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Introduction: Recent studies indicate that patients with an increasing mixed chimerism after allo-PBSCT have a significantly enhanced risk of relapse. However, as long as we now, none of them have focused on RIC transplantation. Aim: To study the relationship between the degree of chimerism and the frequency of relapse, graft rejection, graft-versus-host-disease (GVHD), overall survival (OS) and event free survival (EFS) in patients who have received RIC allo-PBSCT. Patients. 102 consecutive stem cell transplant recipients (RCT) with peripheral blood stem cells from identical MHC sibling donors with reduced intensity conditioning at a single center were included in the study. Their characteristics were: median age 53 (23-69); Male/Female: 64/38; Sex disparity: 50%; Diagnosis: 21 MM, 19 NHL, 17 AML, 11 CLL, 8 HL, 5 ALL, 4 CML, 2 CMPD, 1 CLL+HL. Two patients died prior to be evaluated, while the remaining cases were valuable for acute GVHD (aGVHD). In addition, 78 patients were included in the analysis for chronic GVHD (cGVHD). 77 were analysed by serial and quantitative chimerism analysis at days +28, +56 and +100. The mean follow-up was 385 days (21-2502). Methods. After genomic DNA extraction from bone marrow samples, Powe rPlex™16 System kit (Promega Corporation, Madison, WI) was used to amplify 16 STR regions (15 plus gender marker, Amelogenin). The amplified products were analysed using GeneScan 2.1 (Applied Biosystems, Foster City, CA) after electrophoresis in the ABI Prism 377 (Applied Biosystems). For statistical analysis, the χ² and t-Student tests were used. Log-rank applied to compare differences between survival curves. Multivariate analysis was carried out according to the cox-regres sion method. Criteria to define the chimerism status were the previous ly described by Bader et al (JCO, 2004, 22:1696): Complete chimerism (CC) - No autologous cells at any time after transplantation. Low-level mixed chimerism (LL-MC) - Weak (<5%) autologous signals. Decreasing mixed chimerism (de-MC) - Autologous signals decreasing >5% during follow-up. Increasing mixed chimerism (in-MC) - Autologous signals increasing >5% during follow-up. Results. 56/77 revealed CC or LL-MC; in-MC was found in 15 patients and de-MC in 6 patients. Relapse was significantly more frequent in patients with in-MC (12 of 15) than in patients with CC/LL-MC (14/58) or de-MC (2/6; p<0.001). The probability of 5-years EFS was 41% for all patients, with 7% for patients with in-QM and 51% for the rest patients (p<0.001). Within the 15 patients with in-MC, 6 received additional immunotherapy (DLI or bortezomib). This latter group had a significantly higher 5-year OS (67%) than those who did not receive immunotherapy (11%, p=0.085). Regarding GVHD, patients with CC/LL-MC/de-MC have more incidence of cGVHD (70%) than those with in-MC (15%, p<0.001). Conclusion: Serial analysis of chimerism reliably identifies patients at high risk to relapse. Accordingly, patients with increases MC should be actively treated because they are on high risk of relapse.

SERIAL ANALYSIS OF WHOLE BLOOD AND CD3+ T-LYMPHOCYTE CHIMERISM FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION WITH ALEMTUZUMAB CONTAINING REDUCED-INTENSITY-CONDITIONING

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Serial analysis of haemopoietic chimerism can be used to predict outcome after allogeneic SCT using a RIC regimen and guide post-transplant intervention. Although alemtuzumab is increasingly used as a component of RIC regimens, there have been few studies of its impact on chimerism status post-transplant. We have therefore measured chimerism following allogeneic SCT in whole blood/marrow nucleated cells (WB) and magnetically selected CD3+ T-lymphocytes in 64 patients with lymphoid (8 high-grade NHL, 18 low-grade NHL, 5 myeloma, 3 mantle cell lymphoma, 9 Hodgkin’s lymphoma) or myeloid malignancy (14 AML, 3 MDS, 1 myelofibrosis, 3 CML) conditioned with alemtuzumab and either fludarabine with melphalan (n=46), BEAM (carmustine, etoposide, cytarabine and melphalan) (n=16) or fludarabine/busulphan (n=2). Forty-seven patients received a transplant from an HLA compatible sibling donor and 17 from a matched unrelated donor. All patients achieved neutrophil and platelet engraftment. Donor engraftment was quantified within the first year post-allograft as well as following donor lymphocyte infusions (DLI) by FISH or PCR-based analysis of polymorphic microsatellite regions. 85% of patients demonstrated full donor chimerism (FDC, defined as ≥95% cells of donor origin) in WB within the first 90 days post-transplant. By contrast FDC was only present in the CD3+ compartment of 45% of patients. The proportion of patients with WB FDC declined to 64% by 12 months post transplant whilst the proportion of patients with CD3+ FDC remained constant. Thirteen patients received DLI using escalating CD3+ doses for management of mixed donor chimerism (MC) including 6 with evidence of disease relapse. Following DLI 7 patients achieved FDC in WB and CD3+ compartments and 4 failed to switch to FDC. Seven patients developed acute GVHD post-DLI. Acquisition of FDC in the CD3+ compartment within 90 days post-transplant correlated with the presence of acute GVHD (p=0.005). Sixteen patients relapsed of whom 13 exhibited MC in WB or CD3+ compartments. Three patients relapsed despite the presence of FDC in WB and CD3+ cells. There was a trend towards improved disease free survival in patients who achieved FDC in WB within 90 days of transplantation compared to patients with MC (median 30 months v 11 months respectively). These data define a different pattern of WB and CD3+ chimerism after alemtuzumab regimens compared with T-replete RIC regimens and confirm a correlation between chimerism status and outcome post-transplant.

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CHIMERISM STATUS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION
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With the engraftment of allogeneic transplantation, the patient becomes a real chimera, because of the cohabitation in the same person of a genetic patrimony coming from two different people: the patient (pt) and the donor. The periodic control of chimerism is very important for several reasons. It identifies the cellular population’s type present after transplantation, and the timely identification of a possible disease’s relapse. We have investigated the kinetics of engraftment in 133 pts with different malignancies. Seventy nine (median age: 47 range 22-62) received reduced conditioning regimens: 22 Flu/Mel, 26 Flu/Cy and 29 Flu/TBI and 54 pts (median age: 33 range 10-58) received myeloablative conditioning regimen. We have also evaluated if CD34 cell dose influence engraftment. Due to its high sensitivity, chimerism’s valuation is performed using multiple PCR coamplification of 16 Short Tandem Repeat loci in a single reaction. Donor/recipient cell population ratio was detected by calculating peak area of PCR products for each informative marker. The median number of informative alleles was 2 (range 0-8). We have evaluated the number of patients that have reached the complete chimerism (DCD>C95% donor’s cell) at days +15, +30, +90, +180, +270, +360 and so on. In the subgroups of pts that received non myeloablative conditioning regimens the outcome was respectively: 20/79 (25%), 30/79 (38%), 49/79 (62%), 97/79 (94%). We have also evaluated that engraftment’s kinetics of non myeloablative transplantation is more gradual in time compared to the myeloablative transplantation. In this last one, the engraftment is more rapid and the complete chimerism is already reached on the 30th day. In non myeloablative transplantation, donor engraftment was evaluated at day +15, +30, +90 and so on, in three subgroups of pts that have received different CD34 cell dose:<2×10^6/kg, >2×10^6/kg; and >8×10^6/kg. At the day +15 the kinetics of engraftment resulted significantly correlated to dose (p<0.028), while from the day +30 it didn’t significantly differ in the three subgroups (p>0.5). Conclusions: The valuation of the transplantation’s kinetics of engraftment has shown that in non myeloablative transplantation is normal to have a mixed chimerism with tolerance host versus transplantation and engraftment versus host, for this reason we suggest the importance of the periodic control of chimerism in order to modify the immunosuppressive therapy in favour of the chemotherapy and to identify immediately the disease’s relapse. The CD34 cell dose has a noticeable effect only in the early kinetics donor chimerism (1 to 15 days).

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HEMATOPOIETIC RECOVERY AFTER LOW INTENSITY CONDITIONING TRANSPLANTS AND STANDARD ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)-COMPARATIVE ANALYSIS
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Aim: To compare hematopoietic reconstitution after low intensity conditioning transplants and standard allogeneic hematopoietic stem cell transplantation (HSCT). Methods: We retrospectively analyzed the kinetics of cytopenia of 50 consecutive patients treated with HSCT during a 60 day posttransplant period. Twenty four patients were treated with a low intensity conditioning regimen (Fludarabine, 2 Gy total body irradiation) and 26 patients with the standard conditioning regimen. Patients who received the low intensity HSCT were analyzed in two groups, patients with engraftment of donor hematopoiesis and those who rejected the graft. Results: Patients treated with low intensity conditioning, regardless of its outcome, experienced significantly less severe cytopenia than the patients from the control group. Except for reticulocytes, the development of cytopenia was significantly slower in these patients, and the duration of severe cytopenia was significantly shorter. However, full neutrophil recovery (absolute neutrophil count >1.0×10^9/L) took longer in patients with low intensity HSCT. Conclusions: The kinetics of cytopenia and hematopoietic recovery after low intensity conditioning HSCT significantly differ from standard HSCT. There is no difference in the initial hematopoietic recovery between patients with or without engraftment after low intensity conditioning. This indicates that the onset, severity, and duration of the cytopenia are influenced primarily by the intensity of the conditioning and by the immunosuppressive regimen after transplantation. Effects are more pronounced for neutrophils than for platelets and reticulocytes.

0836
ANTITHYMOCYTE GLOBULIN THERAPY FOR STEROID-RESISTANT ACUTE GRAFT VERSUS HOST DISEASE
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1Gregorio Maran Hospital, Madrid, Spain; 2 Germans Trias i Pujol Hospital, Badalona, Spain
Background and Aims. Acute graft-versus-host disease (aGVHD) is a major cause of mortality after allogeneic stem cell transplantation (ASCT). Initial treatment includes both calcineurin inhibitor therapy and corticosteroids (2 mg/kg/d). Steroid-resistant aGVHD (SR-aGVHD) is considered when progression after at least 3 days of treatment is observed, lack of response after at least 7 days of therapy, or incomplete response after a treatment course of 14 days. SR-aGVHD usually develops in 30-60% of patients, needing secondary intervention using antithymocyte globulin (ATG) as an option of rescue therapy for RS-aGVHD. We report the experience of two Spanish institutions with this therapy. Patients and Methods: 155 ASCT has been performed in both institutions in the last 9 years. 67 patients (43.2%) developed a GVHD which required first line therapy including corticosteroids. A complete response (CR) was observed in 38 of them and 34 patients (51.8%) developed SR-aGVHD. ATG was administered for rescue therapy in 21 of them. The characteristics of patients are shown at Table 1. Results: Our results are presented at Table 2. CMV infection was observed in 12/21 (57.14%) of ATG treated patients. Long term survivors (3/21) developed extensive chronic GVHD. Conclusions: Despite initial clinical improvement (12 responses, 6 of them achieved CR), overall survival was poor in our series (3/21) and one year mortality approached 86% for patients with SR-aGVHD. Only 3 patients are long term survivors, all of them with CR after ATG therapy. It appears that the early use of ATG could improve the SR-aGVHD response rate. Nevertheless, mortality was high due to the lack of response or to opportunistic infections. Of note that 7 NM transplants who developed SR-aGVHD responded to ATG therapy (5 CR, 4 PR).

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| CR: complete remission; NR: no remission; PR: partial remission; LPS: lymphoproliferative syndrome; MRD: graft versus host disease; TTP: thrombotic thrombocytopenic purpura; *: sepsis; #: aGVHD; #: LPS; @: cGVHD; @: relapse; §: TTP.
Background and aim: Cytomegalovirus (CMV) infection remains one of the most important complications for patients (pts) undergone allogeneic stem cell transplantation (allo-SCT). We evaluated incidence and outcome of CMV infection after allo-SCT with reduced-intensity conditioning (RIC) regimen. Methods. 30 consecutive pts (male: female = 17:13) aged from 38 to 67 years (median: 57) were allografted with bone marrow (5) or peripheral blood stem cells (27) from HLA-identical sibling donors from 2000 to 2005. The underlying hematologic malignancies were: acute myeloid leukemia (AML 8), myelodysplastic syndrome (MDS 7), non-Hodgkin lymphoma (NHL 6), multiple myeloma (MM 5), chronic lymphocytic leukemia (CLL 2), idiopathic myelofibrosis (MF1 1) and chronic myeloid leukemia (CML 1). RIC regimens performed were: TT-EDX (17), FLU-TT-EDX (6), TBI 200 cGy (6), and FLU-TBI (1). CMV donor/recipient status was: positive/positive (27), positive/negative (2), negative/positive (1), negative/negative (0). All pts were weekly evaluated with CMV pp65 antigenemia assay for first month; thereafter antigenemia was determined only when a CMV infection was suspected due to clinical or biochemical features. The decision to switch from prophylactic to preemptive therapy was made on the basis of two consecutive positivity or the first positivity > 5/200.000 cells. Acyclovir was given as CMV prophylaxis; pre-emptive therapy consisted of ganciclovir, valganciclovir, foscarnet or cidofovir. Results. A positive CMV antigenemia was detected in 10 pts; all of them were seropositive for CMV before alloSCT. 4 pts had AML, 2 NHL, 1 CLL, 1 MM, 2 MDS. The incidence of CMV infection was 10/30 (33,3%). 7 pts presented only one episode of CMV reactivation, 1 patient two episodes and 2 pts three episodes. Median time of first CMV positive antigenemia was 52 days after alloSCT (range: 22-356), particularly 8 pts had CMV reactivation before 100 days post SCT. Median positive cells at the first appearance of antigenemia was 3/200.000 cells (range 1-14). Overall, we reported 15 episodes of CMV reactivation, 9 before and 6 after 100 days post alloSCT. Pts who developed late CMV reactivation showed contemporaneously chronic GVHD or disease relapse. Only 2/15 (59%) episodes were treated. Anti-CMV drugs employed had similar effectiveness with a median time of CMV clearance of 15 days. None developed CMV disease nor died for CMV infection. 5/10 pts who suffered CMV infection are still alive; while 5/10 died for disease progression (4) or GVHD (1). 13/20 (65%) pts without CMV infection are still alive. In the same years, 33 pts received a myeloablative allo-SCT from HLA identical donor: among them 6 pts developed a CMV infection for an incidence of 18%. Conclusions. in our group of pts transplanted with RIC allo-SCT, the incidence of CMV reactivation was 33.3%. Pre-emptive therapy was effective and no patient developed CMV disease.
Cytokines and growth factors

0839
ERYTHROPOEITIN (EPO) AS AN IMMUNOMODULATORY AGENT: EPO-ASSOCIATED B CELL PROLIFERATION
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1Tel-Aviv University, Tel-Aviv, Israel; 2Institute for Veterinary Physiology, Zurich, Switzerland

Erythropoietin (Epo) is the key hormone regulating erythropoiesis. Reombinant human Epo (rhEpo) is thus used as a major treatment for various types of anemias. Studies in the past decade have revealed extramedullary sites of Epo production, along with abundance of Epo receptors in various tissues and cell lines, suggesting that this hormone may actually have pleiotropic activities. Our previous studies have implicated Epo as an anti-neoplastic agent in murine multiple myeloma (MM) models (Mittelman et al., PNAS 2001; Katz et al., 2005). The implication that CD8 type T lymphocytes are involved in the anti-neoplastic effects of Epo raised the possibility that Epo has a wide-range of immunomodulatory effects. We thus investigated the effect of Epo on the immune system, focusing on two experimental models. (a) Epo-injected mice as compared to their diluent-injected counterparts (b) transgenic mice constitutively overexpressing Epo (termed tg6) as compared to their age-matched wild-type siblings. In both experimental models we found increased B-cell responses related to Epo effects. Namely, Epo-treated and Epo transgenic mice displayed higher proliferative responses to lipopolysaccharide (LPS) in vitro, indicating Epo-associated improved B cell functionality. On the other hand, in-vitro stimulation of splenocytes with T cell specific mitogens (e.g. Concanavalin A and anti-CD3) elicited less proliferation in Epo-treated and in Epo-overexpressing mice, as compared to their control non-treated and wild type counterparts, respectively. In accordance with these data, FACS analysis of splenocytes from the Epo transgenic mice demonstrated a moderate decrease in CD4-positive T cells and moderate increases in the CD19-positive B cells and in Epo transgenic mice displayed higher proliferative responses to CD19-positive B cells and in Epo transgenic mice displayed higher proliferative responses to CD8-positive T cells and moderate increases in the CD19-positive B cells and in Epo transgenic mice displayed higher proliferative responses to

0840
IL-10 GENE POLYMORPHISM INFLUENCE THE CLINICAL COURSE OF NON-HODGKIN’S LYMPHOMA
J. Mazur,1 K. Bogunia-Kubiak,1 T. Wrobel,2 K. Kucilczkowski,1 A. Lange3
1Wroclaw Medical University, Wroclaw, Poland; 2Lower Silesian Centre for Cellular Trans, Wroclaw, Poland;

Backgrounds. Non-Hodgkin’s lymphomas (nHL) are heterogeneous group of lymphoproliferative disorders. In the North America and Europe the most frequent nHL are the B-cell lymphomas. Interleukin-10 (IL-10) is an important anti-inflammatory cytokine, mainly produced by Th2 and B lymphocytes. Many studies have shown that IL-10 may be involved in the pathogenesis of lymphoid disorders. Production of many cytokines is related to its gene promoter polymorphism and this polymorphism could be associated with aggressive form of nHL. We have recently demonstrated that the association between the presence of TGF-1B high producer genotype - TGF-1B +696 T/C (Leu10Pro) / 915 G/G (Arg25Arg) and TGF-1B -696 T/T (Leu10Leu) and 915 G/G (Arg25Arg) - the extra nodal manifestation of the non-Hodgkin lymphoma (Cytokine 2006). Aim: In this present study IL-10 gene polymorphisms were analysed in 55 NHL patients and 50 controls. Methods. IL-10 gene promoter polymorphisms at positions (-1082 A/G, -819 C/T, -592 A/C) were determined by PCR-RFLP technique employing commercial primers (One Lambda, Inc. Canoga Park, CA, USA). Results. Only a slight prevalence of ACC among patients as compared to controls was observed (32/55 vs. 21/50, p=0.07). Interestingly, this genotype was more frequently detected in patients with more aggressive disease (17/23 vs. 15/32, p=0.04) and in those with 2 or more extra nodal sites of the disease (11/14 vs. 21/41, p=0.07). To assess if IL-10 ACC genotype (associated with lower IL-10 production) constitutes an independent risk factor of more aggressive course of nHL (multivariate logistic regression analysis was performed). IL-10 ACC genotype together with other clinical and biological factors (patient sex and age, stage and aggressiveness of the disease, presence of B symptoms, serum LDH level) was subject-
**0842**  
SPONTANEOUS TRANSFORMATION OF LYMPH NODE AND BONE MARROW STROMAL CELLS FROM CANCER PATIENTS  

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^1^1st Medical Faculty, PRaha 3, Czech Republic; ^2^Medical Faculty, Hradecky Kralove, Czech Republic; ^3^University Hospital, Hradecky Kralove, Czech Republic  

**Background.** Until recently, human cells were regarded resistant to spontaneous in vitro transformation. Last year, two papers in Cancer Research and Cytotherapy reported that diploid mesenchymal stem cells (MSCs) can convert to malignant phenotype in vitro in presence of high concentrations of fetal calf serum, AILS. We have obtained our first transformed stromal cell line in 2003. Since then, we have been studying conditions necessary for spontaneous in vitro transformation and in vitro and in vivo properties of transformed stromal cells. **Methods.** Lymph node stromal cells were obtained from patients undergoing diagnostic or curative surgical procedure for lymph node (4 patients) or epithelial cancer (5 patients). Bone marrow MSCs were obtained from patients undergoing diagnostic or staging bone marrow biopsy. After tissue dissection, cells were centrifuged on Ficoll gradient and mononuclear cells were allowed to adhere to tissue culture plastic. Adherent cells were grown in α-MEM with 10% fetal calf serum (FCS) or in α-MEM with 2% FCS supplemented with dexamethasone, ascorbic acid, EGF and PDGF-BB. Surface, cytoplasmatic and nuclear antigens were studied by flow cytometry and immunofluorescence. Cytogenetic analysis was performed after standard G-banding. Transformed cells were injected subcutaneously or intraperitoneally in nude mice and tumors were examined histologically. **Results.** We have obtained transformed stromal cells from all seven lymph nodes grown in α-MEM with 10% FCS. Transformation occurred very quickly, during initial expansion in one case and from 5th to 10th passage in other cases. Only two transformed cell lines were obtained from more than twenty bone marrow aspirates and in both cases, the transformation occurred during 2nd passage. Before transformation, cell cultures did not undergo neither senescent nor crisis phases and normal cells were very quickly overgrown by morphologically abnormal cells with average doubling time of 38 hours. Immunophenotypically, these cells resembled MSCs and were CD90+, CD166+, CD34-, CD45-, cytokeratin- and CD117+. They were also positive for telomerase, grew without contact inhibition and were unable to differentiate into osteoblasts or adipocytes. Transformed cells were hyperdiploid to hypertetraploid (49-115 chromosomes), with nonrandom pattern of chromosomal gains and losses. When administered subcutaneously into immunodeficient animals, these cells produced locally invasive sarcomas and in several cases, visceral metastases were found after intraperitoneal implantation. On the other hand, cells from the same samples grown in MAPC medium with 2% FCS only retained their usual spindle-shaped morphology, contact inhibition, diploid karyotype and ability to differentiate into specialized cells. **Conclusions.** Stromal cells from cancer patients lymph nodes were prone to quick malignant transformation, while mesenchymal stem cells from bone marrow were much more resistant. For transformation, growth medium with 10% FCS was required in both cell types. After transformation, all the cell lines had very similar phenotype, karyotype and clinical behaviour. Whether the easy in vitro transformation is an inherent feature of lymph node stromal cells or reflects the wide-spread genomic instability of cancer patients remains to be established.  

**Funding.** This work was supported by grants IGA 7448/3, MSM 0021620808 and MSM 0024620820.

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**0844**  
EVALUATION OF THE EXPRESSION OF ANGIOTENIC CYTOKINES AND THEIR RECEPTORS IN AUTOIMMUNE MYELOFIBROSIS  

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^1^Bari University Medical School, Bari, Italy; ^2^Department of Hematology, Bari, Italy; ^3^Department of Pathology, Bari, Italy  

**Background.** Autoimmune myelofibrosis (AM) is an emerging clinicopathological entity, resulting in various degrees of isolated or combined chronic peripheral blood cytopenias. It is defined by a pattern including: increased reticulin fibrosis, not clustered megakaryocytes, reactive lymphoid infiltration in bone marrow biopsies; absence of significant tear drop poikilocytosis and leukoerythroblastosis on peripheral blood smears; normal sized spleen; positive autoimmune serology, possibly fulfilling the classification criteria of an autoimmune disease. It has to be distinguished from different conditions associated with myelofibrosis; among these, the most relevant differential diagnosis is with chronic idiopathic myelofibrosis (CIM), particularly when disclosing autoimmune clinical and/or laboratory features. **Aims.** We purposed to assess the bone marrow stromal changes in AM with particular regard to the...
expression of angiogenic cytokines and their receptors, estimation of microvessel density (MVD), and immunophenotype of the lymphoid component. The aim of the present study was to evaluate, by immunohistochemistry, the expression of various isoforms of angiogenic cytokines and their receptors in bone marrow biopsies of AM in comparison with the expression patterns of the same cytokines and their receptors preserved in CIM and normal bone marrows, as described by Chou et al. (Leuk Res 2008; 32: 499) and Yoon et al. (Acta Haematol 2000; 104: 151), respectively. Methods. The tested cytokines and their receptors included platelet derived growth factor (PDGF, PDGFRα, PDGFRβ), basic fibroblast growth factor (bFGF) and its receptors (FGFR1, FGFR2, FGFR3, FGFR4), vessel endothelial growth factor (VEGF) and its receptor (VEGFR1), transforming growth factor β (TGFβ1, TGFβ2, TGFβ3) and its receptors (TGFβR1, TGFβR2). Immunohistochemistry was performed by an immunoperoxidase method with avidin-biotin complex, using specific commercial antibodies (Santa Cruz Biotechnology, USA) on trephine biopsies derived, before treatment, from eight patients (age range: 40-78 years; 6 females) diagnosed as affected by AM. Controls skipping primary antibodies were used as negative controls. Results. The immunohistochemical staining for TGFβR1 on endothelial cells of small vessels, and bFGF on megakaryocytes were markedly decreased compared to those observed in CIM samples. For the other tested cytokines and their receptors, AM samples showed patterns of staining and cellular localization similar to those found in CIM and normal bone marrows. Conclusions. The results of our comparative study suggest that the different bone marrow expression of TGFβR1 in endothelial cells and bFGF in megakaryocytes could be useful to differentiate AM from CIM.

**0845 PROINFLAMMATORY CYTOKINES IN THE PATHOGENESIS OF DENGUE FEVER AND HEMORRHAGIC DENGUE FEVER IN VENEZUELA**

N. Soyano, A. Müller, A.E. Soyano, C. Cruz, E. Olivo
Venezuelan Inst. for Scientific Research, Caracas, Venezuela; Clinica El Avila, Escuela Luis Razzetti, Caracas, Venezuela; Escuela Luis Razzetti, UCV, Caracas, Venezuela; Clinica El Avila, Caracas, Venezuela

Background. Dengue fever is an flu-like acute viral disease highly prevalent in several regions of Asia and America. It is transmitted by the mosquito Aedes aegypti. In around 30% of the patients, the disease progresses towards the haemorrhagic form, which may be potentially life-threatening when haemorrhagic shock develops. The disease has become endemioepidemic in Venezuela, constituting a severe problem of public health. The pathogenesis of the haemorrhagic form of the disease is far from clear, although several cytokines are believed to play an important role. MATERIALS AND METHODS. During the period 2004-2006, especially in the rainy season, numerous cases of dengue fever (DF) were detected in Venezuela. We studied seventy two (72) patients, whose age ranged from 9 to 76 years, were admitted at Clinica El Avila (Caracas, Venezuela) with clinical and laboratory diagnosis of DF. At the time of admission (usually 3-4 days from the beginning of the symptoms), besides the clinical laboratory samples to evaluate routine hematological parameters, coagulation tests (prothrombin time, PTT, thrombin time, fibrinogen and fibrin degradation products, plasminogen and antithrombin), blood chemistry (BUN, creatinine, transaminases), a blood sample was taken to determine plasma concentration of five different cytokines: IL-2 (interleukin-2), IL-6, IL-8 and TNF-α (tumor necrosis factor-α) and GM-CSF. These were quantified with an ELISA assay using a commercial kit (QuantiKine TM, R & D Systems, Minneapolis, MN, USA). Twenty (20) apparently healthy blood donors served as normal controls. RESULTS. Of the 72 patients with DF, 24 patients (34%) showed evidence of haemorrhagic dengue fever (HDF) (platelet count below 100,000/μL and signs of haemococoncentration indicated by an haematocrit greater than 20% of normal value); of these, 11 patients (45.8%) developed petechiae, purpura and severe thrombocytopenia with platelet count below 20,000/μL, requiring administration of plasma components or platelet transfusions. No cases of dengue shock syndrome were observed. Concentrations of TNF-α were found to be significantly increased in 23 patients (31.9%) when compared to normal controls. The increase in TNF-α concentration was positively correlated with the severity of thrombocytopenia. IL-6 was increased only in 11 patients (15.3%), significantly more than the other cytokines and their receptors. IL-2, GM-CSF and IL-8 concentrations were not significantly different from those of the normal controls. CONCLUSION. These data suggest that the development of DF and HDF circulating proinflammatory cytokines such as TNF-α and IL-6 play an important role in the pathogenesis and severity of the disease. Levels of other cytokines such as IL-2, IL-8 and GM-CSF were unmodified.

**0846 EFFECT OF GRANULOCYTE COLONY STIMULATING FACTOR (G-CSF) ON DECREASED IL-12P40 PRODUCTION BY CHEMOTHERAPY**

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Backgrounds. IL-12 is a 70-kDa cytokine comprised of two disulphide-linked proteins (p35 and p40). The highly coordinated expression of p40 and p35 genes to form IL-12 (also called p70) in the same cell type at the same time is essential for the initiation of effective immune response. Granulocyte-colony stimulating factor (G-CSF) affects the balance in the cytokine network, and is believed to facilitate the differentiation of myeloid precursors towards neutrophilic myeloid lineage. In the present study, we investigated the plasma IL-12 p40 and IL-12 Mix production in patients with B-cell lineage NHL treated with chemotherapy (e.g., CHOP regimen) with or without G-CSF administration. Methods. Initially, we examined the plasma IL-12 p40 and IL-12 Mix of the 28 NHL patients before chemotherapy and then at days 10, 17 and 24 after chemotherapy. We confirmed plasma IL-12 p40 (191.2 pg/mL) and IL-12 Mix (277.4 pg/mL) in patients were higher than healthy volunteers (IL-12 p40 76.4 pg/mL, IL-12 Mix 48.5 pg/mL). p<0.04, 0.02. Next, we examined 9 patients with all course of chemotherapy with administration of G-CSF (CG: n=9) and without G-CSF (C:n=9). Serum IL-12 p40 and IL-12 Mix levels with each course were decreased after 10 days chemotherapy, the group of CG were significantly decreased than group C. Serum IL-12 p40 and IL-12 Mix levels in the group of C (IL-12 p40; mean ± SD, from 154.2 ± 121.8 pg/ml to 24.6 ± 27.3 (10 days), 103.8 ± 59.0 (17 days) pg/ml, IL-12 Mix; mean ± SD, from 154.2 ± 156.4 pg/ml to 18.3 ± 17.8 (10 days), 97.5 ± 75.7 (17 days) pg/mL) significantly decreased in comparison with the group of C (IL-12 p40; mean ± SD, from 168.8 ± 77.4 pg/ml to 49.1 ± 43.2 (10 days), 167.8 ± 67.2 (17 days) pg/ml, IL-12 Mix; mean ± SD, from 222.6 ± 95.3 pg/ml to 48.6 ± 44.1 (10 days), 226.6 ± 98.2 (17 days) pg/ml) at 10 days and 17 days after chemotherapy (IL-12 p40; p=0.011 (10 days), p=0.006 (17 days), IL-12 Mix; p=0.0001 (10 days), p=0.0001 (17 days)). These results showed that administration of G-CSF decreased serum IL-12 p40 and IL-12 Mix levels. Interestingly, plasma IL-12 p40 level in CG group patients with clinical stages was and was significantly decreased after chemotherapy than before chemotherapy (mean±SD, -95.6±131.1 pg/ml) (n=16 course) compared with group C (mean±SD, -0.1±35.2 pg/ml) (n=10 course) (p=0.035). However, plasma IL-12 Mix level in CG group patients with clinical stages was not significantly decreased after chemotherapy than before chemotherapy. Plasma IL-12 p70 levels could not be detected in almost all patients. We analyzed the association with survival rate with administration of G-CSF. The overall survival (OS) at 24 months was not significantly differed between both groups (C: 58.3% vs VS GC: 80.0%, p=0.67). However, the survival in the patients of clinical stages and with CG group (n=6) significantly improved than C group (n=4) (stages and survival rate 66.6% vs 25.0%, p=0.02). Conclusions. We found that chemotherapy with G-CSF decreased IL-12 p40 production. We did not find the difference in overall survival at the present study. However, a longer administration of G-CSF might have an influence on the survival rate by reducing an immunosuppressive IL-12 p40 production.

**0847 ANGIOGENIC MOLECULES IN HODGKINS DISEASE: RESULTS FROM SEQUENTIAL SERUM ANALYSIS**

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Backgrounds. The induction of new vasculature from pre-existing vessels, termed angiogenesis, is a prerequisite for tumour growth and metastasis and is controlled by a complex network of angiogenic enhancers and inhibitors. Increased angiogenic activity has been demonstrated in...
lymphoproliferative diseases including Hodgkin’s disease. Aims. The aim of the current study was to measure the levels of circulating angiogenic molecules in Hodgkin’s patients prior to and after treatment and correlate them to disease stage and prognostic score. Patients-Methods. Serum samples were obtained from sixty patients with newly diagnosed Hodgkin’s disease (mean age±SD: 41±19 years) and nineteen healthy individuals (mean age: 59±10 years). Serum samples were obtained from all patients prior to initiation of treatment and in 45 within 6 months of completion of standard ABVD therapy. Six patients relapsed in less than 6 months and 5 died. Two of the 60 patients were diagnosed as Hodgkin’s Ann Abror’s stage I, 42 stage II, 5 stage III, 10 stage IV. International Prognostic Scores (IPS) of 0, 1, 2, 3, 4 and 5 were recorded for 14, 15, 19, 8, 1 and 3 patients. Elisa measurements were performed using the Quantikine, R & D kits (Minneapolis, MN, USA) for human Hepatocyte growth factor (HGF), Vascular endothelial growth factor (VEGF), Angiogenin, Angiopoietin-2, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). Results. Using the non-parametric Mann-Whitney test, there was strong evidence of higher median concentrations in the pre-treatment group compared to controls for TNF-α (20.8 versus 14.9 pg/ml, p<0.001), IFN-γ (1958.8 versus 744.1 pg/ml, p<0.001), IL-6 (14.1 versus 3.1 pg/ml, p<0.0001) and VEGF (794.4 versus 297.4 pg/ml, p=0.001). Angiogenin and angiopoietin-2 levels did not differ from controls. TNF and HGF were found increased in stages III/IV in comparison to stages I/II (p<0.05). No statistically significant differences between patients with low and high prognostic score were detected. HGF and VEGF correlated significantly with IL-6 (r=0.56, p=0.0008 and r=0.57, p<0.0001 respectively). HGF, TNF-α, VEGF and angiogenin decreased significantly following effective treatment (p<0.01). Conclusion. In conclusion, Hodgkin’s disease displays an angiogenic activity as depicted by the increased levels of a number of angiogenic cytokines. HGF seems to be the prominent molecule in Hodgkin’s disease, which may be used to monitor the disease status and the response to treatment.

0848 LEVELS OF CYTOKINES AND OTHER INFLAMMATORY MARKERS IN PATIENTS AFTER ALLOGENIC STEM CELL TRANSPLANTATION


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Objective: Infectious complications remain a major cause of morbidity and mortality after allogeneic haematopoietic stem cell transplantation (HSCT). Diagnosis and outcome might be improved by using early, sensitive and specific laboratory parameters. The aim of the study was observation of dynamics for some inflammatory markers: IFN-γ, TNF-α, interleukin (IL)-18, IL-8, IL-6, serum amyloid A (SAA), C-reactive protein (CRP), procollaciton (PCT) and neopterin measured at the first 25 days after allogeneic HSCT. Methods. We studied 20 patients (mean age: 52.4±10 years) with haematological malignancies and aplastic anaemia undergoing allogeneic HSCT from related donors with different conditioning regimens and 30 healthy controls. Levels of cytokines and neopterin were measured by ELISA method, CRP and SAA immunonephelometric but PCT immunoluminometric method. Results. Major transplant-related complications (MTCs) included bacteremia, veno-occlusive disease of the liver, idiopathic pneumonia syndrome, CMV infection, endothelial leakage syndrome and grade 2-3 acute GVHD occurred in 38% of patients. Compared with other complications those with MTCs developed higher levels of IL-18 (896 vs 359,1 pg/ml) and IL-8 (571.8 vs 17,82 pg/ml) beginning from the first day after HSCT procedure; IFN-γ (41,6 vs 15,6 pg/ml at day +6); IL-6 (295,8 vs 64,9 pg/ml at day +6); CRP (2,41 vs 0,88 pg/ml at day +6 to +8) and neopterin (46,3 vs 10,7 mmol/l on day +6 (p<0,01). Mutual interrelations confirm correlation between increased concentration of neopterin and IL-6 (r=0,274); with PCT (r=0,546); SAA (t=0,522); IFN-γ (t=0,266), TNF-α (t=0,714); p<0,05. Conclusions. IL-18 and IL-8 were shown to be the earliest markers and the best predictors for GVHD after allogeneic HSCT.

0849 TNF-α INDUCES APOPTOSIS IN CELLS UNDER ERYTHROID DIFFERENTIATION

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Backgrounds. Inflammatory cytokines inhibit the proliferation of erythroid progenitor cells, among other effects upon iron homeostasis and erythropoietin synthesis, all of which contribute to the pathogenesis of anaemia, a common complication of chronic diseases. Recent studies suggest that the increased release of TNF-α could be responsible for the development of anaemia through the induction of an apoptotic mechanism mediated by death receptors. It has been suggested that this cytokine effect is dependent on the stage of erythroid differentiation. Aims. The effect of TNF-α upon differentiation, proliferation and apoptosis was investigated in cells subjected to erythroid differentiation. Methods. K562 (erythropoietin-independent) and UT-7 (erythropoietin-dependent) cells were cultured in the presence of haemina (H) for 48 h to study proliferation (Trypan blue test), differentiation (DAF staining) and apoptosis evaluated by apoptotic cells (Hoechst fluorescent nuclear stain) and caspase-3 activity (proteolytic cleavage of chromogenic substrate). mRNA analysis was performed by RT-PCR. Results. After 48 h with H, high levels of haemoglobinized cells were observed (K562: 85%, UT-7: 78%). On the other hand, 30 ng/ml TNF-α treatment did not induce significant changes in the development, maturation and viability of both cell lines. Non-differentiated K562 cells were not affected by TNF-α (11) whereas haemin-treated cells were sensitive to the TNF-α proapoptotic effect (Figure A: H-T vs. H-Ly, p<0.002). This apoptotic action was enhanced by PI3Kinase inhibition with Ly294002 (Fig. A. H-Ly-T vs. H-Ly, p<0.05). The negative effects observed in the presence of TNF-α were dramatically decreased by a previous treatment with anti-TNF neutralizing antibody (Figure B). Only in simultaneous experiments with TNF-α and Ly, UT-7 cells cultured in the presence of erythropoietin and induced to differentiation by haemina were induced to apoptosis. This effect resulted significantly higher than that due to signalling inactivation of the growth factor erythropoietin via PI3Kinase (Apoptotic cells: H-Ly-T 92.2±2.4% vs. H-Ly 68±9.5%, p<0.01). Results of caspase-3 activity measurement at 6 h incubation of cell lysates with chromogenic substrate parallel those of apoptotic K562 cells (Figure B). mRNA levels of Bcl-x, the Bcl-2 related protein that acts as important regulator of cell death, were not modified under the experimental conditions mentioned above. The mRNA of c-FLIP, the suppressor protein of apoptotic signals induced by death receptors, was found diminished in K562 induced to erythroid differentiation but not in UT-7 cells grown under similar conditions. Conclusions. During the process of differentiation, cells become sensitive to proapoptotic action of TNF-α. A decrease in c-FLIP expression would explain the apoptosis produced by TNF-α in K562 cells induced to differentiation since this cytokine effect was not observed in differentiated UT-7 cells with non-altered mRNA c-FLIP levels. Besides, cells with different dependence on the growth factor erythropoietin, analysed under similar conditions of erythroid differentiation, show different sensitivity to proinflammatory cytokines. Protective mechanisms against cellular apoptosis caused by TNF-α seem to be mediated by PI3Kinase signalling and proved to be independent from Bcl-x. These findings may have potential implications in the understanding of the mechanisms underlying anaemia in chronic inflammatory diseases.
0850

DEVELOPMENT OF MALIGNANCIES IN MICE TREATED WITH G-CSF FOR A LONG TIME

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Backgrounds. G-CSF is well recognized as a potent mobiliser of hematopoietic stem cells from the bone marrow into the blood, and is being accepted as a regulator of immune responses also. During recent years the use of peripheral blood instead of bone marrow as a source of stem cells has been increasingly employed in the allogeneic transplant setting. A number of adverse experiences concerning donors treated with G-CSF for stem cells mobilization proved it as a safe procedure. However, the parameters used for the safety evaluation have been rather crude. Few cases disclosing several morphological and cytogenetic changes in hematopoietic cells as well as temporal deregulation of some genes in healthy donors were published. Moreover the immunologic complications and leukemia development were noted in G-CSF treated donors. Aims. The goal of this investigation was to study the consequences of several courses of G-CSF treatment using low non-mobilising doses on mice model. Methods Female mice (CBAXC57B16) F1 and (DBA/2xBalb/c) F1 12-16 weeks old were injected subcutaneously with G-CSF (25 mcg/kg) for 4 days once a month with blood cell count and cytology measured before and after the course, another group of mice of the same strains were injected with G-CSF (5 mcg/kg) for 20 days per month with blood cell count and cytology measured monthly. G-CSF courses have been repeated monthly for half a year. After the termination of treatment blood cell count and cytology were performed in all groups once a month. Total observation time was 21 months. The survival rate was evaluated in experimental and control groups. Results During 20 months of follow up 24 out of 40 G-CSF treated mice died due to unknown cause, 8 mice developed different hematopoietic or other malignancies and disorders and were sacrificed. All control mice were healthy with stable number of leukocytes and hemogram. Four-day treatment with 25 mcg/kg/d of G-CSF didn’t change the number of leukocytes significantly, while in the group treated for 20 days with a 5 mcg/kg/d the number of leukocytes had slightly increased. Most of the experimental animals had considerable reticulocytosis. Within the first 7 months of follow up among detected disorders myeloproliferative disorder had dominated, afterwards solid tumors were also detected. Most of animals became neutropenic before disease manifestation. All mice injected with G-CSF for 20 days a month developed pus inflammations in site of injection after 3 months of treatment. Summary/Conclusion. Long-term treatment of animals with low doses of G-CSF (total 100 mcg/kg per month while mobilizing dose for mice is approximately 1500 mcg/kg) may induce malignant transformation and leads to significant decrease of life-span. Mobilization of hematopoietic stem cells with G-CSF is known to promote deregulation of several genes expression which returns to normal profile within 2 months. Perhaps prolonged administration of G-CSF may induce malignant transformation and deregulation of stem cell mobilization. Therefore, G-CSF should be used with caution, especially when used for a long-term treatment of hematopoietic stem cells in healthy volunteer individuals.

0851

PALIFERMIN IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES UNDERGOING AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION (AUTO-HSCT) - PRELIMINARY RESULTS

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Background. Oral mucositis (OM) is a frequent complication of myeloablative therapy and HSCT with no effective treatment. In this multi-center study we tested the ability of palifermin (recombinant human keratinocyte growth factor) to reduce the incidence, duration and severity of OM induced by high-dose chemotherapy followed by auto-HSCT in patients with hematologic malignancies. We also evaluated the requirement for analgesics and parenteral nutrition administered because of OM, incidence of febrile neutropenia (≥38°C), severe infections and requirement for additional antibiotics. Moreover, the influence of palifermin on the hematopoietic recovery after autoHSCT was assessed in this study. Methods and Results. Fifty-six patients with hematologic malignancies were enrolled to the study. Twenty-eight of them (50%) received palifermin (60 microg/kg/day) for three consecutive days before and three consecutive days after conditioning therapy. The median age of the palifermin and control group was 38.3 (range, 19 to 58) and 37.9 (range, 19 to 64), respectively. OM was assessed daily after autoHSCT according to the World Health Organization (WHO) scale. The incidence of OM of WHO grade 1+ was 62.5 percent in the palifermin group and 96.4 percent in the control group and grade 3-4 was 3.5 percent in the palifermin group and 32.1 percent in the control group. Among all patients the median duration of OM was 2.9 days (range, 0 to 11) in the palifermin group and 5.1 days (range, 0 to 27) in the control group. As compared with control, palifermin was also associated with significant reductions in the use of analgesics (21.4 percent vs. 71.4 percent), opioid analgesics (10.7 percent vs. 53.3 percent) and parenteral nutrition (8.5 percent vs. 28.5 percent). No significant differences in the incidence of febrile neutropenia, severe infections and requirement for additional antibiotics were observed between groups. Also palifermin did not impaired reconstitution of the hematopoietic system (Table 1). The drug was generally well tolerated. Adverse events, mainly rash, pruritus, erythema, generalized oedema, mouth/tongue thickness and discoloration, taste alteration and proteinuria were mild to moderate in severity and were transient. Conclusions. Palifermin administration significantly reduced the incidence, severity and duration of OM and did not have negative effect on engraftment in the patients with hematologic malignancies after autoHSCT.

Table 1.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Median (days)</th>
<th>Range</th>
<th>Control group</th>
<th>Median (days)</th>
<th>Range</th>
</tr>
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<tbody>
<tr>
<td>Palifermin</td>
<td>15.8</td>
<td>9 to 42</td>
<td>15.2</td>
<td>8 to 34</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>16.3</td>
<td>8 to 45</td>
<td>18</td>
<td>8 to 54</td>
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</table>

0852

EFFECTIVE MOBILIZATION BY PEG-FILGRASTIM PLUS ARA-C CONTAINING REGIMEN IN PRETREATED LYMPHOMAS PATIENTS

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Background. Studies performed on mice and healthy human volunteers have shown that a single dose of pegfilgrastim (Peg-G-CSF) is effective in stimulating peripheral blood stem cells (PBSC) mobilization. Aims. The aim of this study was to evaluate the efficacy of pegfilgrastim, in combination with salvage chemotherapy, in mobilizing CD34(+) stem cells into the peripheral blood of pretreated lymphoma patients. Methods: We studied 27 pretreated patients (Hodgkin’s lymphomas=5; non-Hodgkin’s lymphomas=22). The median age was 57 years (range 17-70). The patients received a median of 2 previous chemotherapy regimens. Median time from mobilization to harvest was 11.5 days. The efficacy of the mobilization procedure was tested in lymphoma patients receiving salvage regimen [DHAP (cisplatin 100 mg/m², cytarabine 2000 mg/m² × 2) in 17 or MAD (cytarabine 2000 mg/m² × 2 x 5 days in 10) plus pegfilgrastim in 11 or filgrastim in 16. Pegfilgrastim was given as single subcutaneous injection (6 mg) on day +5 post chemotherapy. Filgrastim was given daily (10 µg/Kg) from day +5. Daily monitoring of circulating CD34(+) cells was started from day 8 after the end of chemotherapy. Results. Twenty five/27 patients reached the target cell dose of 2.5×10^6 cells/kg. A median of 2 apheresis (range 1-3) was performed. In pegfilgrastim group, a median of 5.27×10^6 CD34(+) cells/kg (range 1.06-10) was collected. In filgrastim group, a median of 11×10^6...
CD34(+) cells/kg (range 0.09-32.84) was collected. No statistical difference (p=0.06) between the two groups (Pegfilgrastim vs. Filgrastim) was found (Table 1). Conclusions. Our results show that pegfilgrastim as an adjunct to HiDARAC based chemotherapy is an effective mobilization regimen in pretreated lymphoma patients also effective as filgrastim based regimen. This approach is to be confirmed in larger series of patients and probably with increased dose of pegfilgrastim and could open new opportunities in stem cell mobilization for poor or non-mobilizers patients with malignant lymphomas.

<table>
<thead>
<tr>
<th>Table 1. Pegfilgrastim vs Filgrastim in 27 pts (51 apheretic procedures).</th>
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<tbody>
<tr>
<td>Pegfilgrastim</td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>No. apheresis procedures</td>
</tr>
<tr>
<td>Median day to 1st harvest</td>
</tr>
<tr>
<td>Median CD 34×10⁶(overall 8,79)</td>
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<tr>
<td>Poor mobilizer (&lt; 2,5 CD 34×10⁶) overall 6 pts (22%)</td>
</tr>
<tr>
<td>Very poor mobilizer (&lt; 1 CD 34×10⁶) overall 3 pts (11%)</td>
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(>2,5 CD 34×10⁶) overall 21 pts (78%).

LOW DOSE LENGRASITIM IS AS EFFECTIVE AS STANDARD DOSE IN SHORTENING NEUTROPHIL ENGRAFTMENT TIME FOLLOWING MYELOABLATIVE CHEMOTHERAPY AND PERIPHERAL BLOOD PROGENITOR CELL RESCUE

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Backgrounds. G-CSF is widely used following HDT and PBPCR to reduce neutrophil engraftment time. The dose and duration required to gain maximum clinical and economic benefit has not been fully investigated. Aims. This double blind placebo-controlled randomised trial was performed to determine whether short course low dose or standard-dose L would influence recovery of haematopoiesis following HDT and PBPCR. Methods. 61 patients (pts) with non-Hodgkin lymphoma (40) or Hodgkin lymphoma (21) undergoing HDT were randomised between May 1999 and November 2004. Pts had normal peripheral blood counts prior to HDT (Hb ≥100g/L, total white cell count ≥3×10⁹/L, neutrophils (N) ≥1.0×10⁹/L and platelets ≥50×10⁹/L), and had a minimum 2.5×10⁹/L CD34+ cells/kg PBFC previously collected following mobilisation with Cylophosphamide 8 g/m² and G-CSF. All received HDT with BCNU 300 mg/m² d-1, Etoposide 200 mg/m² od d-5-d-2, Cytosine arabinoside 200 mg/m² bd d-5-d-2 and Melphalan 140 mg/m² d-1 before return of PBFC on d0. Pts were allocated standard dose L 263 µg daily (20 pts), low dose L 105 µg daily (21 pts) or placebo injections (20 pts). These commenced on day +5 following PBPCR and continued until N≥0.5×10⁹/L. Pts received standard supportive care including prophylactic Fluconazole and Acyclovir, but not routine antibacterial prophylaxis, until haemopoietic recovery. Results. L at any dose resulted in a significantly shorter median time to N recovery ≥0.5 (10.0 vs 11.0 days, p=0.02) and ≥0.5 (11.0 vs 14.0 days, p=0.005) compared to placebo. The only significant difference between standard- and low-dose L was in hospital stay (21.0 vs 22.0 days, p=0.04), however L at any dose showed a significant reduction over placebo (22.0 vs 23.0 days, p=0.01). There was no significant difference in blood product support or antibiotic usage between the groups. At a median follow up of 40 months there were 27 confirmed lymphoma relapses and 26 deaths (21 relapsed lymphoma, 1 secondary AML, 4 other). Conclusions. Short course low dose L is as effective as standard dose in reducing neutrophil engraftment time following HDT and PBSCR. L at any dose reduces hospital stay when compared to placebo. This approach should be considered for those patients in whom growth factor support is indicated.

MODULATION OF PROTEIN TYROSINE PHOSPHATASE 1B BY ERYTHROPOIETIN IN UT-7 CELL LINE

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Backgrounds. The central role played by tyrosine phosphorylation of erythropoietin receptor (EpoR) in cell activation by erythropoietin (Epo) has focused attention on protein tyrosine phosphatases (PTPs) as candidates implicated in the pathogenesis of the resistance to therapy with human recombinant Epo. The prototypic member of the PTP family is PTP1B, a widely expressed non-receptor PTP located both in cytosol and intracellular membranes via its hydrophobic C-terminal targeting sequence. PTP1B has been implicated in the regulation of a number of signaling pathways, in particular, those involving tyrosine phosphorylation induced by growth factors, cytokines and hormones such as the downregulation of EpoR and insulin receptor. Binding of ligand to cell-surface EpoR results in the activation of JAK2 and phosphorylation of tyrosine residues in the cytosolic domain of the receptor. Termination of the EpoR signaling is attributed to the cytosolic SH-PTP1. However, it has been demonstrated that PTP1B also participates in down-regulation of the ligand-activated cell surface EpoR. Aim. To investigate the effect of Epo on PTP1B expression. Methods. The UT-7 human cell line was used as an Epo-dependent model. Epo was added to serum- and Epo-deprived cells for previous 18 h. After different periods of Epo incubation, cells were lysed and total proteins and RNA were obtained. cDNA was prepared from different RNA samples and PTP1B mRNA level was examined by Real Time PCR. Total proteins or immunoprecipitates with anti-PTP1B were subjected to Western Blot using anti-PTP1B or anti-PTyr. Immunoprecipitates were also subjected to a PTP1B activity assay with pNPP. Then, the experiment was repeated including pretreatment with LY294002 (PI3K inhibitor) before Epo stimulation. Results. An increased and maximum level of PTP1B mRNA was already observed at 3 h of Epo stimulation (figure a). This increment correlates with the induction of PTP1B expression observed by Western Blot (figure b). However, after 9h with Epo, mRNA level returned to baseline while protein expression remained constant. PTP1B Tyr phosphorylation was detectable after 5 min of Epo stimulation and declined within 6 h of PTP1B activity increased after 3 h of Epo incubation and diminished to the basal level within 6 h (figure C). Figure d shows that the pretreatment of UT-7 cells with LY294002 downregulated PTP1B expression in a dose-dependent manner reaching the highest inhibition at 100 mM LY concentration. Conclusions. We have found an Epo-induced expression of PTP1B, associated with increased PTP1B Tyr phosphorylation, suggesting that besides modulating Epo/EpoR signaling, PTP1B suffers a feedback regulation by Epo.
Although plasma cytokine levels (interleukin (IL)-2, IL-4, IL-5, IL-10, tumour necrosis factor (TNF) a and interferon (IFN)-γ) in 164 healthy infants were assessed using the BD Cytometric Bead Array (CBA) kit because it allows the simultaneous measurement of multiple cytokines from small sample volumes. Simultaneously, lymphocyte subsets and classical laboratory parameters like leucocyte count, CRP and immunoglobulins were quantified using the BD cytometric bead array (CBA) kit because it was very valuable for the clinical-therapeutic monitoring of immunological status in various childhood diseases, rather scarce data are available about normal plasma cytokine profiles in the first years of life. Furthermore, while vaccination is an artificial way to introduce protective immunization, no data are available on the potential impact of vaccinating infants on plasma cytokine profiles. In this study, plasma cytokine levels in normal infants without any infections and to evaluate the impact of vaccination on these parameters. Methods. Th1/Th2 plasma cytokine levels (interleukin (IL)-2, IL-4, IL-5, IL-10, tumour necrosis factor (TNF) a and interferon (IFN)-γ) in 164 healthy infants were assessed. Results. In line with these results, lymphocyte subsets and classical laboratory parameters like leucocyte count, CRP and immunoglobulins were determined. Group comparisons were compared with the Mann-Whitney U test (nonpaired Wilcoxon test). Results. Our results showed no statistically significant difference in cytokine levels between T8 and T26 (p<0.05), except for IL-4 (p=0.015). In phase II, T-lymphocytes (p<0.001) T-lymphocytes and IgE plasma levels (p=0.001) were elevated, reflecting a normal developing immune system. Conclusions. As far as we know, this is the first report describing plasma cytokine levels and the potential impact of vaccination on such a large number of healthy infants. For that reason, our values might be very useful in studies on the normal ontogeny of immune cells during infancy. Furthermore, our data can be utilized as age matched references values of cytokine production, which are extremely important for correct interpretations in clinical-therapeutic monitoring of infants during various childhood diseases.

**References**


**Evaluation of the effects of the CD33-targeted drug gemtuzumab ozogamicin on growth and histamine release in human mast cells and basophils**

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**Objectives.** Mylotarg (gemtuzumab-ozogamicin=GO) has recently been introduced as a novel CD33-targeting drug in clinical hematological therapy. However, despite clinical efficacy in acute myeloid leukemia, GO produces significant side effects including an infusion-syndrome. We have recently shown that mast cells (MC) and basophils (BA) express CD33. In the present study, we investigated the effects of GO on mediator secretion and growth of MC and BA. Methods. Growth-inhibitory effects of GO on neoplastic MC (HMC-1) and BA (KU812) as well as cord blood-derived MC- and BA progenitor cells were determined by counting cell numbers and the numbers of apoptotic cells. The amount of histamine secreted from primary MC and BA were measured after incubation of cells with GO alone or GO together with an anti-IL-3 antibody. Results. MC and BA as well as HMC-1 cells and KU812 cells were found to express CD33 mRNA and the CD33 protein. GO was found to inhibit the growth of HMC-1 cells and KU812 cells as well as SCF-dependent differentiation of MC and IL-3-induced growth of BA from

**Monocyte chemotactic protein-1 (MCP-1), also known as CCL2, a chemokine that regulates migration and infiltration by monocytes/macrophages, belongs to the CC chemokine superfamily. Interleukin-8 (IL-8), also known as CXCL8, a proinflammatory chemokine with angiogenesis-promoting properties belongs to the CXC chemokine superfamily. Both MCP-1 and IL-8 have important roles in the pathogenesis of many chronic inflammatory disorders, including atherosclerosis and obesity. Their proinflammatory effects are mediated mainly by the CC chemokine receptor 2 (CCR2) and CXC receptor 1 (CXCR1), respectively. MCP-1 and IL-8 cause vascular inflammation and induce thrombosis, proliferation and migration of vascular smooth muscle cells, angiogenesis, and oxidative stress. Previous studies indicate that: 1) MCP-1 production from endothelial cells, smooth muscle cells, and regional leukocytes increases in the presence of endothelial dysfunction and atherosclerosis risk factors; 2) MCP-1 and IL-8 expression is increased in atherosclerotic lesions and injured arteries; and 3) elimination of MCP-1 function decreases neointimal hyperplasia after injury and atheroma formation in mice. We studied the association between MCP-1 and IL-8 levels and the degree of inflammation in 15 athletes that participated in the ultra-distance foot race of the 246 Km ‘Spartathlon’. This race consists of continuous, prolonged, brisk exercise. We reported earlier significant increases in MCP-1 and IL-8 levels in elite marathon runners. We investigated the effects of GO on mediator secretion and growth of MC and BA. Methods. Growth-inhibitory effects of GO on MC and BA were measured by means of a multi-analyte Biochip Array Technology, using the Evidence analyzer (Randox Laboratories, UK). The measurements were performed before (phase I), at the end (phase II) and 48h post-race (phase III). Results. MCP-1 levels at phase I (216.9 ± 48.5 ng/L) (mean±SE), increased significantly at phase II (592.9 ± 115.7 ng/L) and subsequently decreased at phase III (278.1 ± 62.7 ng/L). At the same time period, IL-8 followed a similar pattern (phase I: 9.4 ± 4.5 ng/L, phase II: 28.5 ± 8.8 and phase III: 8.9 ± 4.3 ng/L). A significant positive correlation between MCP-1 and IL-8 was found at phase III (r=0.845, p<0.01), while this correlation was absent in the other two phases, indicating an independent response of each chemokine to inflammatory stimuli in each athlete. In conclusion, prolonged exercise induces an inflammatory response that is expressed by an increase in circulating MCP-1 and IL-8 levels. Whether these changes have long-term negative effects on the vasculature remains unknown.
their cord blood-derived progenitors. The GO-induced inhibition of growth of neoplastic cells failed to be associated with stress-induced apoptosis. GO neither induced secretion of histamine from MC or BA nor did GO upregulate the anti-IGV-induced release of histamine in these cells. Conclusions. GO counteracts cell growth in normal and neoplastic human MC and BA without inducing release of histamine. Therefore, GO may be considered as a new targeted drug for the treatment of high-grade MC- and BA neoplasms.

0859 ALTERATIONS IN GENE EXPRESSION IN MURINE LEUKEMIA CELLS DEVELOPED AFTER G-CSF TREATMENT
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Background. Four-day treatment of mice with low (25mcg/kg) G-CSF doses is known to be insufficient for mobilisation of hematopoietic stem cells into peripheral blood almost halves the content of bone marrow primitive hematopoietic stem cells and doesn’t affect the CFU-S number. Female mice (CBAXC57Bl6) F1 12-16 weeks old were subjected to such a course once a month. MFD-like myeloid leukemia with histiocytic sarcoma occurred in one case after 3rd course of G-CSF treatment. Liver tissue was totally substituted by undifferentiated cells with no morphologically definable features. The liver was about 4-5-fold enlarged by sight. Bone marrow and liver cells of the mouse were fully transplantable, recipients became moribund within 17-32 days since cells injection. All ill animals had enlarged liver (M 3,4 ± 0,5 g versus normal 1,38 ± 0,3 g). The developed leukemia was not of virus origin, which was proved by three independent methods. Aims. To understand molecular regulation of malignization, differentially expressed genes of interest must be identified, cloned and studied in detail. Methods. Subtracted cDNA library from bone marrow cells of normal and leukemic mice was prepared by Suppression Subtractive Hybridization (SSH) method. Several up-regulated genes were further studied by RT-PCR in bone marrow and liver of leukemic mice. Normalization factor was evaluated by 3 housekeeping genes (HPRT1, RPL13A, UBC) by Genorm software. Results. The clinically ill mice showed a moderate extent of anemia and reticulocytosis, which was supported by suppressed b-globin expression (top 5 down-regulated genes turned out to be b-globin genes). The expression level of c-abl and G-CSF doubled in bone marrow of leukemic mice compared with the normal bone marrow, while the concentration of CFU-C per 105 cells increased 4-fold (247 ± 31,2 in ill mice versus 57,9 ± 27,0 in control animals, p< 0.01). The expression level of genes regulating cell proliferation did not change dramatically - only C-Myc expression increased 3-fold, however concentration of early hematopoietic precursor cells (LTIC) decreased about 5-fold (0,75 versus 3,32 per 105 cells in healthy mice). The pronounced changes were revealed in expression of MPO gene (3,4-fold increase). The level of ill mice consisted of undifferentiated cells. As CD45 expression increased up to 11-fold simultaneously with constitution of liver parenchyma by tumor cell, one may suggest hematopoietic origin of invading cells. CFU-C were also revealed in affected liver (52,5 ± 7,7 per 105 cells). There were minor changes in G-CSF expression in liver cells of leukemic mice, whereas expression of G-CSF-R increased 18-fold compared with normal liver cells. Expression of c-abl also increased. Expression of anti-apoptotic genes was elevated up to 4-fold for bcl-2 and 2-fold for cIAP2. Unlike in the bone marrow, expression of JunB in the liver increased 5-fold. Summary. The G-CSF treatment may lead to development of myeloid leukemia with dramatically changed gene expression and high ability to invade liver tissue.

0860 A SINGLE FIXED DOSE INJECTION OF PEGFILGRASTIM TO MOBILISE AUTOLOGOUS STEM CELLS OF EXTENSIVELY PRE-TREATED LYMPHOMA AND MYELOMA PATIENTS; NOT ALWAYS SUCCESSFUL
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Backgrounds. In patients with multiple myeloma and refractory or relapsed lymphoma consolidation high-dose chemotherapy combined with stem cell rescue is an established therapy in chemosensitive disease. A commonly used approach to mobilise CD34 positive hematopoietic stem cells into the blood is the administration of granulocyte colony-stimulating factor following a course of chemotherapy. Pegfilgrastim, the pegylated form of filgrastim, is subject to a distinct method of clearance by neutrophil leukocytes compared to filgrastim. Pegfilgrastim showed in earlier series of patients to be effective in mobilising blood progenitor cells in single fixed doses of 6 mg, as well as 12 mg. The optimal dose and scheduling of the injection and apheresis is not completely established in various patients groups with diverse extents of chemotherapeutic and radiotherapeutic pre-treatment. Aims. The primary aim was to study the feasibility of a single low dose of pegfilgrastim in extensively pre-treated patients. Methods. Forty-six Consecutive patients with myeloma or relapsed/refractory lymphoma who underwent stem cell mobilisation by identical apheresis techniques using either filgrastim or pegfilgrastim were retrospectively studied. Patient- and disease characteristics, pre-treatment data and mobilising chemotherapy type as well as cytokine dose, apheresis results and neutrophil recovery data were compared. Results. Stem cell harvest was performed in 24 patients after administration of pegfilgrastim once (6 mgs.) and in 22 patients after filgrastim. The filgrastim was administered in a median total dose of 4,2 mgs. (7,7 mgs./kg/day) and 11 injections were needed. The apheresis took place after 13 and 13,5 days respectively, with a maximum CD34-count of 8×10^6/L (pegfilgrastim group) and 11,6×10^6/L (filgrastim group). Of 24 patients who received pegfilgrastim, 5 patients showed a failure mobilising stemcells (21%). In 2 of those 5 patients harvesting succeeded eventually after additional stimulation with filgrastim and another 2 patients were mobilised in a later stage after an additional course of chemotherapy using filgrastim in high dosage. None of the filgrastim mobilisation procedures failed. A median of 7,2×10^6/kg CD34+ cells was obtained, 11×10^6/kg and 6×10^6/kg in myeloma patients. In the filgrastim treated group more CD34+ cells were obtained, 11×10^6/kg, in fewer procedures. The collected number of CD34+ cells per kg bodyweight per ml of processed volume during the apheresis procedure was higher in the filgrastim group, 382×10^6/kg ml (pegfilgrastim) vs. 803×10^6/kg ml (filgrastim). Conclusions. A fixed dose of pegfilgrastim (6 mg) is not sufficient to achieve adequate stemcell mobilisation in all patients. Failure was observed in 21 % of the patients. The number of CD34+ cells collected and the efficiency of the apheresis procedure appear to be higher in the patient group treated with filgrastim.
CIRCULATING VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND ITS SOLUBLE RECEPTORS VEGFR-1 AND VEGFR-2 IN PATIENTS WITH LYMPHOID MALIGNANCY

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Backgrounds. Vascular endothelial growth factor (VEGF) is the most important proangiogenic factor involved in normal and pathologic angiogenesis. Biologic functions of VEGF are mediated by the activation of 3 structurally homologous tyrosine kinase receptors: VEGFR-1, VEGFR-2 and VEGFR-3. The exact role of VEGF receptors in the pathogenesis of lymphoma remains unknown. Aims. The aim of the study was to compare the concentrations of VEGF, VEGFR-1 and VEGFR-2 in the serum of 80 never-treated Non-Hodgkin’s lymphoma patients in different stages of the disease [35 of these patients were diagnosed with the aggressive lymphoma and the remaining 45 patients with the indolent lymphoma; the control group consisted of 17 patients with persistent chronic lymph node enlargement]. Methods. Serum VEGF, sVEGFR-1 and sVEGFR-2 levels were determined by means of the enzyme-linked immunosorbent assays (ELISA) (R&D Systems, USA) according to the manufacturer’s protocol. Results. The VEGF serum concentration was found to be significantly higher in the aggressive lymphoma group when compared to the control group (median=433 pg/mL and 231 pg/mL, respectively; p=0.02026). In the indolent lymphoma group the VEGF concentration was also higher than in the control group, showing a tendency towards statistic significance (p=0.057392). There was no significant difference as far as VEGF between the two studied lymphoma subgroups. The serum concentrations of soluble VEGFR-1 were significantly higher in patients with both forms of lymphoma when compared to the control group (median=86 pg/mL and 44 pg/mL, respectively; p=0.005318). The serum concentrations of the soluble form of VEGFR-2 were significantly higher in patients with the aggressive lymphoma when compared to the indolent lymphoma patients (median=10853 pg/mL and 8985 pg/mL, respectively; p=0.005801). We have not found a correlation between the serum level of VEGF and the soluble forms of its receptors VEGFR-1 and VEGFR-2 in any of the lymphoma patients. We have also checked the ratio as far as the amounts of VEGF and its soluble receptor (activity index VEGF/s-VEGFR-1). Conclusions. The results obtained for VEGF in patients diagnosed with the non-Hodgkin form of lymphoma confirm the role this protein plays in the pathogenesis of lymphoma, especially of its more aggressive form. The higher concentration of VEGFR-2 in the aggressive lymphoma patients when compared to the indolent lymphoma patients shows that - apart from the VEGF concentration - also the concentration of its receptors (especially its second receptor) has an influence on the course of lymphoma. This shows that not only VEGF but also its receptors should be the aim of the antiangiogenic therapy. To sum up, concentrations of VEGF and its VEGFR-2 may have an important influence on the course of Non-Hodgkin’s lymphoma.

INCREASED MT1-MMP EXPRESSION IS INVOLVED IN G-CSF-INDUCED MOBILIZATION OF HUMAN CD34+ HEMATOPOIETIC PROGENITOR CELLS

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Backgrounds. G-CSF is the most established agent for hematopoietic progenitor cell mobilization in clinical practice. G-CSF-induced mobilization is initiated by activation of neutrophils, which secrete various matrix metalloproteinases (MMPs) and serine proteases. These soluble enzymes degrade bone marrow (BM) extracellular-matrix (ECM) and modulate cytokines and receptors, leading to a disruption of cell-cell and cell-matrix interactions and, ultimately, release of progenitors. Yet, progenitor mobilization by G-CSF was apparently normal in mice lacking these soluble enzymes. Therefore, we hypothesized that membrane type 1-matrix metalloproteinase (MT1-MMP), a membrane-bound MMP, might be also required for progenitor egress. MT1-MMP is a key enzyme for normal cell motility and tumor cell migration and invasion. Methods and Results. We found that human CD34+ cells express variable surface MT1-MMP levels, depending on the cell source and G-CSF treatment. The highest expression was found on CD34+ cells enriched from BM and mobilized peripheral blood (MPB) of G-CSF-treated donors (mean fluorescence intensity>900 and 159±40, respectively). MT1-MMP expression was lower in CD34+ cells isolated from the BM of untreated healthy human donors (80±19), and human cord blood (41±4). G-CSF treatment in vitro increased two-fold membranal MT1-MMP expression as compared to IL-6- or SCF-stimulated or untreated human CD34+ BM cells from healthy donors (steady state BM). Importantly, in vivo progenitor mobilization by five daily injections of G-CSF was accompanied by increased MT1-MMP mRNA and protein levels in both mouse BM mononuclear cells and human hematopoietic mature and progenitor cells in pre-clinical model of NOD/SCID mice engrafted with human hematopoietic cells. Immunocytochemical analysis of human CD34+ cells plated on hyaluronate-coated cover slips revealed that in response to SDF-1, MT1-MMP changes its localization in the polarized and spreading cells, suggesting a role in the process of progenitor directional migration. Indeed, blocking MT1-MMP function by antibody (Ab) or its endogenous inhibitor-TIMP-2 slightly but significantly reduced the in vitro chemotactic response of human MPB-derived CD34+ cells through uncoated transwell filters. The effect of MT1-MMP neutralization was even more prominent (60% inhibition) on the CD34+ cell chemotaxis via Matrigel, i.e., ECM barrier. Importantly, in vivo administration of human-specific function blocking MT1-MMP Ab in the course of G-CSF treatment of NOD/SCID chimeric mice almost completely abrogated G-CSF mobilization of human maturing CD45+ leukocytes, immature CD34+ cells and the more primitive CD34+/CD38-low progenitor cells. Finally, analysis of samples obtained from peripheral blood of 29 patients with lymphoid malignancies treated with chemotherapy and G-CSF revealed bone marrow (BM) extracellular-MMP expression (RQ-PCR) and measured MMP activity in the number of mononuclear cells and CD34+ progenitors on the day of first apheresis. Conclusions. We suggest that following G-CSF treatment, increased levels of MT1-MMP on the surface of human progenitors in the BM facilitates their mobilization most probably due to pericellular ECM degradation and/or activation. Our data indicate that MT1-MMP plays an essential role in clinical mobilization procedures, and might serve as a target molecule for new approaches to enhance the mobilization efficiency.

ROLE OF SONIC HEDGEHOG FOR REGULATING THE PROLIFERATION, MIGRATION AND DIFFERENTIATION OF HEMANGIOBLAST IN THE MICROENVIRONMENT OF AGM REGION

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Background. Recently, it was reported that the intra-embryonic aorta-gonad-mesonephros (AGM) region exclusively and autonomously generated hemangioblasts for hematopoietic and endothelial system and dramatically increased hemangioblasts numbers thereafter. It is logically believed that the microenvironment of this region implicated in the
Differential Expression of P-Glycoprotein, But Not of MRP, LRP and BCRP in Leukemic Stem Cells as Compared to More Differentiated CD34+ AML Blasts

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Leukemic Stem Cells (LSCs) retain the hematopoietic stem cell properties of self-renewal, high proliferative capacity, predominant quiescent cell cycle status and differentiation potential, but is biologically distinct from more differentiated blasts. Quiescence confers protection from genotoxic stimuli to LSC, thus contributing to leukemia perpetuation. Currently, it is unknown whether the multidrug resistance (MDR) phenotype may also contribute to the LSC behavior. We analyzed the expression of the MDR transporters: P-glycoprotein (P-gp), MDR-Related Protein (MRP), Lung-Resistance Protein (LRP) and Breast Cancer Resistance Protein (BCRP) in LSCs from 20 CD34+ Acute Myelogenous Leukemia (AML) patients. We restricted our analysis to CD34+ cases, since high P-gp expression has been associated with this phenotype. Protein expression was measured by flow cytometry on LSCs, phenotypically defined as CD34+, CD38−, CD123+ cells, and compared to the more mature CD34+CD38+CD123− blasts. The expression levels were analyzed using: 1) the Kolmogorov–Smirnov test, categorizing D value for each D value as: a) high if D > 0.50, low if D < 0.20; and 2) the mean channel fluorescence index (MFI), defined by the ratio between the mean channel of fluorescence (MCF) for each antigen and MCF of the respective isotypic control. LSCs represented 0.12% (0.02-0.87%) of the leukemic blasts. We observed a distinct expression between LSC, MRP, LRP and BCRP D values in CD34+ blasts and LSCs. Based on the LSC analysis, 95%, 85% and 60% of AML patients were found to have high expression of MRP, LRP and BCRP, respectively. Whereas based on the CD34+ blasts, 90%, 80% and 60% of the patients were thus categorized. Accordingly, MFI for MRP, LRP and BCRP on the two cell subsets were not statistically different (MRP: 176.6 versus 153.3, LRP: 56.1 versus 71.3 and BCRP: 19.5 versus 22 on CD34+ and LSCs, respectively). On contrast, P-gp expression was distinct in LSCs and CD34+ blasts: the mean ± SEM of the D values were 0.38 ± 0.04 and 0.18 ± 0.04 (p<0.006), whereas MFI values were 22.9 ± 5.7 and 11.2 ± 5.9 (p=0.021) in LSCs and blasts, respectively. To our knowledge, this is the first study to show the overexpression of P-gp in the LSCs. Considering that P-gp-mediated drug efflux is the best characterized cellular mechanism of MDR, its constitutively high expression in AML LSCs may give rise to a genotoxic-protected stem cells theoretically capable of perpetuating leukemia.
During fetal life, the spleen is capable to sustain erythropoiesis. We carried out a transcriptional analysis on human UCB hematopoietic cells. To understand FS stromal microenvironment, we identified hematopoietic progenitors that are present in the early stages of development (between 14.5 to 15.5 dpc). Methods. In the FS, we have isolated a CD4int lineage negative (Lin-) population by cell sorting and analyzed its in vitro and in vivo hematopoietic potentials. By limiting dilution assays and clonal assays, the frequency of B, NK, T and myeloid potentials were assessed. We tested the capacity of injected FS CD4int Lin- cells to reconstitute Rag2gc-/- mice. The use of RAG2-GFP mice, the CD4int Lin- population was further characterized. Moreover, by quantitative RT-PCR, we compared gene expression between FS CD4int Lin- population and other progenitors. Results. The FS CD4int Lin- population possesses lymphoid and myeloid potential in vitro. This population keeps its hematopoietic capacities in vivo since CD4int Lin- cells are able to reconstitute the lymphoid and the myeloid compartments of Rag2gc-/- mice. The CD4int Lin- population could be subdivided into three subsets depending on the level of Rag2 expression (Rag2+, Rag2low and Rag2high). The Rag2 subsets are mainly composed of myeloid precursors and we have shown that the loss of the myeloid potential is concomitant to the up-regulation of the Rag2 expression. By clonal assays, we displayed that the Rag2low population is enriched by T/NK progenitors whereas the Rag2hi is mainly restricted toward the B lineage. After 4 days of FS organ cultures, CD4int cells are disappearing while lymphocytes are appearing suggesting that FS lymphocytes derived from in situ differentiation. Moreover, we have determined that the CD4int Lin- population are also the progenitors of the FS CD4hi lymphoid tissue inducing cells that may play a role in the FS architecture. Conclusion. The FS CD4int Lin- population encloses several progenitors that are engaged towards different lineages. These progenitors certainly give rise to committed hematopoietic cells in situ, indicating that the FS actively sustains the lymphoid.

**0868**

**DIMINISHED PROTEASOMAL DEGRADATION RESULTS IN ACCUMULATION OF GFI1 PROTEIN LEVELS IN MONOCYTES**

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**Backgrounds.** Gfi1 is a transcriptional repressor essential during myeloid differentiation. Gfi1-/- mice exhibit a block in myeloid differentiation resulting in the accumulation of an immature myelomonocytic cell population and the complete absence of mature neutrophils. Even though mRNA levels of Gfi1 appear to be very low in monocytes, Gfi1 might play a role in the monocytic lineage as Gfi1-/- mice exhibit diminished monocyte-derived dendritic cells and disturbed cytokine production by macrophages in response to LPS. Aim. Study the role of Gfi1 in monocyte differentiation. Methods. Gfi1 mRNA and protein levels were measured by qPCR and Western blot analysis respectively. Modifications of Gfi1 with ubiquitin were analyzed using His-tagged ubiquitin and binding of Gfi1 to gene promoters was analyzed with chromatin immuno-precipitation assays. Results. Upon forced monocytic differentiation of U937 cells, Gfi1 mRNA levels dropped but protein levels increased indicating that Gfi1 protein expression is mainly regulated post-transcriptionally. To study this we performed ubiquitination experiments and found that Gfi1 is efficiently targeted by the ubiquitin-proteasome pathway. Treatment of cells with proteasome inhibitors MG132 or Velcade resulted in significant increases of both transfected as well as endogenous (U937) Gfi1 levels. Remarkably, after PMA induced monocytic differentiation of U937 cells proteasome inhibition did not result in an increase in Gfi1 levels. In line with this, we found that radioactive labeled in vitro translated Gfi1 was rapidly degraded in lysates taken from U937 cells, a process which could be blocked by proteasome inhibition. When lysates were taken from PMA stimulated U937 cells, the Gfi1 turnover was significantly delayed. Thus, during PMA forced differentiation of U937 cells Gfi1 protein levels rise due to diminished degradation. Similar findings were found in primary cells. Gfi1 mRNA levels were low in primary monocytes while the protein was clearly detectable. Conversely, Gfi1 mRNA levels were high in granulocytes but the protein was swiftly degraded by the proteasome in these cells. Chromatin immunoprecipitation experiments showed that Gfi1 binds to the promoter of several granulocyte-specific genes in primary monocytes, including C/EBPa, neutrophil elastase and Gfi1 itself. The binding of the repressor Gfi1 to these promoters correlated with low expression of these genes in monocytes compared to granulocytes. Conclusions. Gfi1 undergoes efficient ubiquitin-proteasomal degradation in immature hematopoietic cells. Upon monocytic differentiation proteins levels increase due to diminished proteasomal degradation, despite low RNA levels. Our data fit a model in which Gfi1 protein levels are induced in primary monocytes to repress genes that play a role in granulocytic differentiation.

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**HIGHER CONSTITUTIVE NF-kB SIGNALING IS A DISTINCTIVE FEATURE OF UMBILICAL CORD BLOOD CD34+ PRECURSORS**

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**Background.** Delayed engraftment, better reconstitution of progenitors, higher engraftment function, and a lower incidence of the graft versus host disease (GVHD), are characteristics associated with umbilical cord blood (UCB) transplants when compared to bone marrow (BM). These differences are in part due to the action of distinct genes and pathways among hematopoietic stem and progenitor cells (HSPC) of these two sources. Aims. We carried out a transcriptional analysis on human UCB and BM HSPC, in order to identify the molecular differences and factors responsible for their control. Methods. Pools of CD34+ positive cells sorted by immunomagnetic methods (MACS) with over 92% purity were used to obtain RNA from HSPC of both sources. Transcriptional analysis was carried out by serial analysis of gene expression (SAGE). Differential expression of selected genes was evaluated by real-time PCR on additional CD34+ samples from BM (n=22), UCB (n=9) and G-CSF mobilized peripheral blood (MPB, n=6). Results. We sequenced approximately 60,000 tags from each SAGE library, roughly corresponding to
10,000 genes. Although HSPC from BM and UCB where very similar, a stringent statistical analysis revealed a set of 61 tags (transcriptors) differentially expressed, 45 overrepresented in UCB and 16 in BM. The set of UCBCoverrepresented genes included both subunits (NFkB2 and RELB) of the NF-κB transcription factor complex involved in the sustained activation of NFκB transcription targets, trough the non-canonical constitutive pathway. In addition, factors such as interleukin 1 (IL1A and IL1B), lymphotactins receptors and tumour necrosis factor (TNFRSF18, TNFRSF4), known to induce and sustain non-canonical NFκB signaling, were also found. Higher expression of transcripts coding for activators (IL1B, TNF and TGB1), effectors (NFkB2, RELA and RELB) and transcriptional targets (ICAM1, IL8 and CCL4L) of NF-κB signaling were found in UCB HSPC and confirmed by real-time PCR. Finally, the expression of the genes over-expressed in UCB HSPC revealed a statistically significant overrepresentation of NFκB cis-regulatory elements, including known NFκB transcriptional targets genes (such as CXCL2, CXCL5, ICAM1, IL8, ILB, NFkB2 and RELB), and novel potential targets of NFκB signaling (like RGS1, zyxin and others). NOTCH1, which controls the transcription of NFκB, was also overrepresented in UCB. Conclusions. Our results point out to a central role of the NF-kB pathway on the molecular and functional differences observed between BM and UCB HSPC. Moreover, NFκB inhibition is known to cause apoptosis and loss of clonogenic function in HSPC. Furthermore, UCB HSPC readily differentiates into T cell on fetal thymic organ cultures, while BM HSPC must be pre-treated with TNF. Thus, NFκB transcription targets and other UCB overrepresented genes such as MIP1B, MIP2A, MIP2B, IL8, IL1, RGS1, zyxin, ICAM1, TGBF, LTβ, TNF, may be responsible for the differences related to cell survival, quiescence, mobility and adhesion of these cells, as well as increased T cell diversity and evasion of this central mechanism set the basis for future studies and to potentially new strategies to stem cell graft manipulation to improve the outcome of transplants with these cells, as well as their handling on propagation cultures.

CLOSE FUNCTIONAL SIMILARITIES BETWEEN HUMAN MESENCHYMAL STEM CELLS, PERICYTES AND FIBROBLASTS

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Backgrounds. Mesenchymal stem cells (MSC) are pluripotent precursors capable of differentiating into osteoblasts, adipocytes and chondrocytes, present in bone marrow (BM) and in various other adult and fetal tissues. MSC share many properties with pericytes, which form a contiguous, although spatially variable network in the vascular bed. Whether name stromal cell or fibroblast is often used interchangeably with MSC. Aims. To evaluate how similar are MSC obtained from different human tissues and what is their relationship with fibroblasts and pericytes on the basis of their gene expression and functional properties. Methods. A set of 30 genes was selected on the basis of previous results of gene expression analysis (SAGE) for MSC, CD34+ cells and fibroblasts, and their expression was examined in 20 different human cell lines cultured in vitro: 7 MSC from different adult and fetal sources, 2 in vitro differentiated MSC cultures (one differentiated into osteoblasts and one into adipocytes), 4 of fibroblasts, and one of pericytes (isolated from the human retina), bulk bone marrow, endothelial cells, liver cells, brain tissue, skeletal muscle and heart tissue, respectively. Cells were characterized by their immunophenotype by flow cytometry, and the capacity to differentiate into osteoblasts, adipocyte and chondroblasts. Gene expression of selected genes was measured by real-time PCR or semiquantitative RT-PCR. Results. All MSC and the pericyte culture had the capacity to differentiate into osteoblasts, adipocytes and chondroblasts, whereas fibroblasts did not differentiated under similar conditions. Cluster analysis of gene expression profiles using Name 4.0 showed that all the MSC lines formed a very close cluster which included pericytes and fibroblasts, separated from other normal human cells. In addition, all the MSC lines and pericytes had similar immunophenotypic markers and capacity for in vitro differentiation. Similarity of the gene expression profiles of MSC and fibroblasts were further confirmed by clustering of the 1,000 top expressed tags of SAGE libraries for 21 normal human tissues. Despite the similarity, genes related to angiogenesis, especially CXCL6, were more expressed in MSC from umbilical vein, from adults saphenous vein and in pericytes. Differentiation into adipocytes or osteoblasts was accompanied by the increased expression of specific genes, although the global patterns were still very similar, so that they remained in the same cluster together with the MSC. Conclusions. MSC that can be obtained from a variety of adult and fetal tissues and organs have very similar immunological markers, differentiation potential and gene expression profiles. Comparison of these characteristics also shows that human MSC, pericytes, and fibroblasts are very closely related, representing probably different functional states of the same cell. Identity between MSC and pericytes is particularly striking, whereas fibroblasts seem to have lost most of their differentiating potential. These results have practical as well as conceptual applications, since they demonstrate the functional equivalence of pericytes and MSC from different origins, and their close relationship to fibroblasts.
mechanisms implicated in T-ALL, and especially related to TAL-1. Methods. Four to five NOD/SCID mice, previously irradiated with 325 rads, were injected with 10 or 20 millions leukemic cells. When possible, positively separated CD34+ cell precursors were also injected. Injections were done intra-venously, intra-peritoneally or directly into the bone marrow. Results. We have transplanted 5 T-ALLs characterized by different oncogenic abnormalities (TAL-1, LM02, HOX11L2) in NOD-SCID mice. So far 3/5 T-ALL samples induced a leukemia whatever was the injection route (intra-venous, intra-peritoneal or intra-bone injection). Two experimental groups are still on going. Human leukemic cells were found in the peripheral blood, bone marrow, spleen, thymus and lymph nodes. However the spleen was the site, which contained the highest number of leukemic cells. In 2/2 T-ALL samples secondary transplantations were performed and induced a leukemia that allowed tertiary transplantation. The immunophenotype of the engrafted leukemic cells in primary and secondary mice was heterogeneous but still contained cells phenotypically identical to the original transplanted sample, especially when recovered from the mice bone marrow. In spite of the modification of the immunophenotype of leukemic cells, the serial transplantation results are evidences of the presence in the T-ALL samples of self-renewing leukemic stem cells. Transplantation of limiting numbers of total leukemic cells or of a CD34+ positive leukemic cell population are ongoing in order (1) to quantify the frequency of T-ALL initiating cells in samples and (2) to delineate the phenotype of a leukemic stem cell population. Conclusions. This in vivo model will enable the study of the molecules involved in the development of T-ALL and particularly in the molecule that cooperate with TAL-1 in this pathology.

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MESENCHYMAL STEM CELLS ARE EFFECTIVE AT PREVENTING BUT NOT TREATING GRAFT-VERSUS-HOST DISEASE

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Background. Evidence has emerged that mesenchymal stem cells (MSC) represent a promising population for cellular therapy. MSC are of stromal origin and can differentiate into multiple lineages, including osteoblasts, chondrocytes, adipocytes, neurons and skeletal myocytes. MSC also possess immunosuppressive properties, which make them particularly attractive to control unwanted immune responses. For this reason they have been used in allogeneic haematopoietic stem cell transplantation to manipulate graft-versus-host disease (GvHD). We wanted to test the ability of MSC to prevent and/or treat GvHD in a xenogeneic model. Methods. After sublethal irradiation, nonobese diabetic (NOD)/severe combined immune-deficient (SCID) mice were transplanted with 20×10⁶ CFSE-labelled human PBMC obtained from normal buffy coats. Mice also received MSC generated from cord blood at the time of PBMC infusion or at the onset of xenogenic-GvHD (x-GvHD). Recipient mice were evaluated at serial intervals for: 1. human T cells proliferation measured by CFSE staining and number of CD45+CD3+ cells; 2. clinical signs of x-GvHD (wasting, ruffled hair, hunched back). At the end of the experiment lymphoid and non-lymphoid tissues were examined by histological analysis. Results. The human PBMC-NOD/SCID chimera monitored at different weeks after injection of PBMC showed extensive human T cells proliferation in their peripheral blood which was initially evident at 5 weeks. The mice started to develop signs of x-GvHD after 8-10 weeks and the disease was then confirmed by histology. Lymphoid infiltrates were evident not only in lymphoid tissues but also in liver, kidney, spleen, lung and pentothal washed. The chimeric mice injected with a single dose of MSC at the time of PBMC infusion did not behave differently from the controls. However, when MSC were given at weekly intervals, there was a marked decrease in human T cells engraftment and none of the mice developed x-GvHD. If MSC were administered when x-GvHD had already developed, no difference in T cells expansion and course of the disease was observed as compared to controls. Conclusions. Our study shows that systemic administration of MSC in human PBMC-NOD/SCID chimera dramatically increases the survival of the animals in a dose-dependent fashion. Human T cells proliferation and x-GvHD-induced tissue damage are markedly reduced in the treated mice and the use of MSC alone appears to be safe and well tolerated in this model. This work supports the clinical use of MSC infusions in SCT as a prophylactic treatment of GvHD.

0874

THE SIDE POPULATION MAY PROVIDE THE LEUKEMIC STEM CELL COMPARTMENT COMPLEMENTARY TO THE CD34+CD38- STEM CELL COMPARTMENT. IMPLICATIONS FOR STEM CELL MDR DETECTION AND THERAPEUTIC TARGETING


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Backgrounds. Acute myeloid leukaemia (AML) is a haematopoietic stem cell (HSC) disease. Although chemotherapy is initially successful in the majority of AML patients, many patients relapse, suggesting that chemotherapies are ineffective at eliminating the leukemic stem cells (LSCs). Although the AML CD34+CD38- compartment is enriched for LSCs, not all LSCs can be CD34+CD38-, e.g. in CD34 negative AML. An alternative stem compartment is the so-called side population (SP). SP cells are defined by their ability to efficiently efflux Hoechst 33342 dye and in normal bone marrow (nBM) are enriched for HSC activity. In AML, the SP compartment is able to initiate leukaemia in NOD/SCID mice. The exact immunophenotype and the relationship with the CD34+CD38- stem cells are largely unknown. Aim: to define the immunophenotype of AML SP cells in relation to nBM SP cells. Such information may enable AML SP stem cell detection at all stages of disease and ultimately guide stem cell directed therapies. Methods. Using Hoechst dye and antibodies against CD34, CD38, CD7, CD19 and CD56 (the latter three offering leukaemia associated phenotypes, LAF used for MRD detection), as well as C-type Lectin-like molecule-1 (CLL-1), a marker for the AML CD34+CD38- compartment (van Rhenen et al, Blood 2005; 106: 4), SP immunophenotyping was performed on HM from nBM samples of AML patients at the time of diagnosis. Results. The 8 AML patients had a median SP frequency of 0.01% (range 0.003-0.17%). For the immunophenotype, in terms of CD34 and CD38 expression, we found i) the whole blast compartment was partly CD34+CD38+ and partly CD34+CD38- in the 5/6 CD34 positive (>1% CD34) cases and almost completely CD34-CD38- in the 3 CD34 negative cases; ii) in 5 CD34+ cases SP cells were in majority CD34+CD38+; the rest was mainly CD34-CD38-; iii) also in the 3 CD34- cases CD34+CD38- was the predominant phenotype (located in the very small CD34- compartment); iv) in all cases there was only a very small CD34-CD38- compartment (median 4% of SP cells). LAFs present on the whole blasts were also present on the SP cells in all 6 LAP+ cases, indicating malignancy. SP cells from nBM samples were completely LAP negative. FISH analysis in a (8;21) AML patient confirmed SP malignancy. In all 8 cases SP cells were partly or completely positive for CLL-1. SP cells from nBM were completely CLL-1 negative. Summary/Conclusions. Our results suggest that the phenotype of AML SP cells not necessarily reflects that of the whole blast compartment and in addition to being reported as CD34+CD38- or CD34-CD38-, in most cases is CD34+CD38-. CLL-1 and LAP expression on AML SP cells, similar to CD34+CD38+ stem cells, offers the ability for stem cell detection under MRD conditions and especially for negative cases of AML. In addition, SP cells might play a role in the CD34+CD38- stem cell compartment. In addition, CLL-1 expression on both AML SP and CD34-CD38- LSCs and not on normal HSCs offers the ability for potentiation of toxic coupled antibody stem cell therapies now covering all known AML stem cell phenotypes.

0875

HUMAN UMBILICAL CORD BLOOD CELLS REGENERATE HEPATOCYTES IN A NON-MYELOABLATIVE SETTING


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Backgrounds. A number of reports have shown that rodent bone marrow cells can transdifferentiate into hepatocytes. Human umbilical cord blood, a rich source of haematopoietic stem cells and mesenchymal progenitor cells which might be used for tissue or organ repair. Aims. We evaluated whether human umbilical cord blood cells infused following non-myoablative conditioning can regenerate hepatocytes after acute liver injury in an immuno-competent mouse model. Methods. In an acute hepatic injury model, female C57Bl6 mice were administered toxic doses of acetaminophen. Six hours later, the mice were given sulfadiazine (0.5 mg/kg) and cyclosporine (5 mg/kg) followed by infusion of human umbilical cord blood mononuclear cells at a dose of 1×10⁷ mononuclear cells per kilogram of body weight. The cyclosporine was
continued at 3mg/kg daily for four more days. Surviving mice were sacrificed at two and four weeks post-transplant. Fluorescence in-situ hybridization (FISH) and polymerase chain reaction (PCR) analysis of hepatic DNA for a-satellite region of human chromosome 17 were used to confirm the presence of hepatocytes from human origin. Results. Fifteen out of 24 mice received umbilical cord blood cells infusion after non-myeloablative conditioning survived beyond two weeks compared with two of the 11 control mice (p=0.027). The surviving mice were sacrificed at two weeks and four weeks post-transplants. Histological sections showed regenerating liver with hepatocytes of normal appearance comparing with the livers of the control mice showing extensive necrosis. FISH analysis confirmed the presence of human Y-chromosome positive cells in the hepatic sections of all but three of the surviving mice, the percentages ranged from 0.5-9%. PCR analysis showed that all except 3 mice (lanes 9, 12 and 15) showed presence of about 1.5-20% human DNA (Figure). In three mice (lanes 6, 14 and 15), about 10-20% of the hepatic DNA was of human origin. There was a concordance between the proportion of human DNA detected by PCR and the percentage of human Y-chromosome positive cells by FISH. Non-hepatic tissue (heart and kidney) did not contain human DNA. Conclusion: Our data suggested that human umbilical cord blood could repair acetonaphen induced acute hepatic injury in a non-myeloablative setting. Our model closely mimics the clinical UC transplantation setting and should be further explored in a clinical setting. In the future, this may be an effective approach in the management of patient with fulminant hepatic failure waiting for orthotopic liver transplantation.

**Figure 1. Detection of human DNA in the livers of mice survived.**

**0876**

TOWARD STANDARDIZATION OF CELLULAR PRODUCTS FOR IMMUNOMODULATION AND REGENERATIVE MEDICINE. EXPANSION OF MESENCHYMAL STEM CELLS DERIVED FROM AMNIOTIC FLUID: PERSPECTIVES OF FUTURE CLINICAL APPLICATION

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**Background.** Mesenchymal stem cells are now extensively studied in projects involving either their immunomodulatory property and their utilization in regenerative medicine. Because of the limited absolute number of bone marrow (BM) derived MSC available as cell therapy product we addressed our attention to MSC derived from alternative sources. Recently, it has been suggested that amniotic fluid (AF) is a rich source of MSC. Aim. In the current study, we evaluated the isolation expansion of AF-MSC testing the immunophenotype and karyotype stability at different passages. The expansion of MSC derived from BM and AF were compared. Methods. Cell isolation from AF and BM. Second trimester samples of AF were centrifuged for 10 minutes at 400g. Cells were plated in Mesencult medium and, after 48 hours, non adherent cells were discarded. The expansion of AF-MSC was assessed plating 5 × 10⁵ cells at four different density since the first passage. Bone marrow aspirates were obtained from posterior iliac donor volunteers. BM mononuclear cells (MNC) were isolated, plated in 75 cm² flasks in Mesencult medium and incubated at 37°C with 5% humidified CO₂ atmosphere. Flow cytometry analysis: at each passage, surface expression of CD14-APC, CD34-PE, CD45-PerCP, CD31-PE, CD73-PE, CD105-PE, HLA-A-APC, HLA-B-PE was determined. Results. MSC from AF could be extensively expanded in vitro showing immunophenotype similar to that of BM-MSC. Starting from 95 mL (median value) of BM aspirate we were able to obtain a median number of 2.5 × 10⁸ MSC at first passage (P1) up to 65.34 × 10⁶ MSC (range 4.6-90 × 10⁶ and 9.9-144 × 10⁶ respectively) at fourth passage (P4). Using the lowest plating density, we optimized the fetal MSC expansion, achieving the highest number of expanded AF-MSC (median number 1.2 × 10⁶ and 2 × 10⁶ cells at P4 and P5 respectively).

Conclusions. AF-MSC showed higher expansion capability when compared to BM derived MSC. The lowest cell plating density represents the best condition to promote AF-MSC expansion. Although this condition seems to boost cell growth, we didn't find any karyotype abnormality on all the samples tested. AF-MSC could represent a potentially very useful cell therapy product.

**0877**

INTRACORONARY AUTOLOGOUS BONE-MARROW STEM CELL TRANSFER AFTER MYOCARDIAL INFARCTION. PRELIMINARY RESULTS OF A RANDOMISED TRIAL


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**Backgrounds.** Recent experimental and clinical studies have shown that cardiac transfer of stem cells and progenitor cells derived from bone marrow may enhance functional recovery after acute myocardial infarction (AMI). Aims. To assess whether intracoronary transfer of autologous bone-marrow cells could improve left ventricular remodelling and global ejection fraction after 6 months' follow-up. **Methods.** A total of 40 patients with AMI, were randomly assigned to either a control group (n=20) that received optimum postinfarction medical treatment, or a bone-marrow-cell group (n=20) that received optimum postinfarction medical treatment and intracoronary transfer of autologous bone-marrow cells in the first week after the primary percutaneous coronary intervention. Autologous bone marrow stem cells were obtained by iliac crest aspiration. After density gradient centrifugation, mononuclear cells were incubated for 24h in Teflon bags with X-vivo medium at 37°C. Prior to intracoronary infusion, cells were washed and resuspended in 10 mI normal saline. We assessed left ventricular volumes and function from baseline to a minimum of 6 months' follow-up by cardiac magnetic resonance imaging. **Results.** Median volume of bone marrow aspirate was 36 ml (range 80-40), with a total number of mononuclear cells of 157 million (65-400). The number of infused cells was 80 million (20-215), with a viability of 90 ±12%, and a recovery rate of 59 ±19%. The content of CFU-GM and BPU-E was 45 ±21 and 150 ±106 per dish, respectively. No infusion related complications were observed. Global left ventricular ejection fraction (LVEF) at baseline was 43.7% ±14.2 in controls and 48.8% ±12.7 in the bone-marrow-cell group (p=0.26). Functional evaluation after 6 months is available so far in 25 patients (11 controls and 12 treated with bone marrow cells). Mean global LVEF in controls was 49.3% (p=0.25) and 46.15% (p=0.64) in the bone-marrow-cell group. No significant differences in ventricular volumes were found between both treatment groups. **Conclusions.** In patients with marked left ventricular dysfunction after AMI, we haven’t found significant improvement of LVEF in the bone-marrow-stem-cell group after 6 months follow-up. The potential impact on long-term survival needs further evaluation.


**0878**

LOCAL INJECTION OF BONE MARROW CELLS AUGMENTS THE NEOVASCULARIZATION IN A MOUSE ISCHEMIC HIND LIMB BY INDUCING VEGF

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**Backgrounds.** Improved neovascularization is an important therapeutic goal after myocardial infarction and limb ischemia. In the recent years, increasing evidence suggests that bone-marrow derived circulating cells (BMCs) in the ischemic sites might augment angiogenesis and collateral vessel formation. Aims. We examined whether the BMCs might induce the angiogenesis as effectively as endothelial progenitor cells (EPCs) in a mouse model of hind limb ischemia and evaluated the expression of related molecules. Methods. BMCs and EPCs were isolated from C57BL/6 mice and were labeled with PKH26 (10×10⁶ cells/animal). Unilateral hind limb ischemia was surgically induced by femoral artery ligation in C57BL/6 mice (control group; n=4), autologous BM-MNCs (Group 1; n=4, 1.8±0.2×10⁶/animal), and EPCs (Group 2; n=4, 1.1±0.21×10⁶/animal) were transplanted into the ischemic limbs after 10 days. After 4, 8, 12 weeks, the capillary/muscle ratios were evaluated. And VEGF, eNOS, ProMMP-9 and MMP-9 were assayed in tissue homogenates using western blot. Results. Injected PKH26 labeled BMCs were observed for 12 weeks and the expression of VEGF by IHC. The capillary/muscle ratios were evaluated. And VEGF, eNOS, ProMMP-9 and MMP-9 were up-regulated than control. The expression of MMP-9 was normalized within 14 days after BMCs injection while the elevated expression of VEGF was sustained after 12 months (Figure 1). So enhanced vascularization by BMCs is thought to be related with the upregulated expression of VEGF. Conclusions. This result suggested that direct local transplantation of autologous BMCs augments the neovascularization in ischemic tissues. And prolonged expression of VEGF could eventually participate in blood vessel formation.

![Figure 1. The expression of VEGF by IHC.](image)

**0879**

ABNORMALITIES OF BONE MARROW MESENCHYMAL STEM CELLS IN PATIENTS WITH CHRONIC IDIOPATHIC NEUTROPENIA

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**Backgrounds.** Chronic idiopathic neutropenia (CIN) is an acquired underproduction neutropenia syndrome characterized by hypoplastic and left-shifted granulocytic series in the bone marrow (BM). Previous studies have shown that the bone marrow (BM) microenvironment may contribute to the pathophysiology of the disease by producing pro-inflammatory cytokines and being a depot for defective support of granulocytepoiesis. Whether, however, there is a primary defect at the mesenchymal stem cell (MSC) level in these patients remains unknown. Aims. To study the reserves, the functional characteristics and the differentiation potential of BM MSCs in patients with CIN. Methods. Thirteen patients with CIN and 22 age- and sex-matched healthy controls were studied after informed consent. All patients had neutrophil counts below 1800/microliter and were satisfying the previously reported diagnostic criteria for the disease. The BM mononuclear cells (BM-MNCs) were isolated from posterior iliac crest aspirates and the MSCs were expanded according to a standard protocol. MSCs were characterized by their immunophenotypic characteristics (CD45−,CD34−,CD14−,CD73−,CD44−,CD29−,CD105−,CD146−) and their adiogenic (Oil red O stain and aP2 and PPAR-γ expression by RT-PCR), osteogenic (ALP/Von Kossa stain and ALP and CBFA1 expression by RT-PCR), and chondrogenic (Masson and Alcian blue stain and Collagen II and aggrecan expression by RT-PCR) potential after induction of differentiation in appropriate media. The frequency of MSCs in the BMNC fraction was evaluated by means of a limiting-dilution assay (LDA) based on the Poisson probability. The functional characteristics of MSCs were studied by evaluating (a) their clonogenic potential using a standard colony forming unit-fibroblast (CFU-F) assay and enumerating the CFU-Fs/1000MSCs plated through passages (P); (b) their proliferative potential by using the MTT assay and evaluating the cell doubling time (2ⁿ=cells counted/cells plated) in each passage. Results. CIN patients displayed normal number (14.64±14.53 MSCs/10⁶cells) and normal immunophenotypic characteristics of BM MSCs. The clonogenic, osteogenic and adiogenic potential of patient MSCs did not differ from the respective of the controls as was evaluated by the collagen II and aggrecan, the ALP and CBFA1, and the aP2 and PPAR–mRNA expression, respectively, by means of a semi-quantitative RT-PCR. Compared to healthy controls, however, patient MSCs displayed impaired CFU-F potential time-course (P<0.001; P1-P6) as well as impaired proliferative capacity. This was demonstrated by the MTT assay (P<0.01 at P1) and the cell doubling time-course (P<0.001; P1-P7). Summary/Conclusions. Patients with CIN display normal number and differentiation potential of BM MSCs. The clonogenic and proliferative potential of patient MSCs, however, is defective compared to the respective of the healthy controls. Analysis of the production of growth factors and inhibitors at protein level as well as the telomeric length of patient MSCs is currently under investigation to elucidate further the pathophysiology of the observed MSC abnormalities in CIN patients.

**0880**

CONGENITAL NEUTROPENIA: A GROUP OF DISORDERS WITH GENETIC AND PHENOTYPIC HETEROGENEITY

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Severe congenital neutropenia (CN) is a general term for a group of disorders characterized by extremely low blood neutrophil counts (ANC<0.5×10⁹), early stage maturation arrest of myelopoiesis, and recurrent
Superior Effects of High-Dose Enzyme Replacement Therapy in Type 1 Gaucher Disease on Bone Marrow Involvement and Chitotriosidase Levels: A Two-Center Retrospective Analysis

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Background. Gaucher disease type I is the most common lysosomal storage disorder, caused by deficient activity of the enzyme glucocerebrosidase (OMIM *606465), leading to the accumulation of glucocerebroside in spleen, liver and bone marrow. The most important clinical manifestations are hepatosplenomegaly, cytopenia and skeletal involvement. Gaucher disease can be treated with enzyme replacement therapy (ERT), leading to a dramatic clinical response in most patients. However, even after more than 14 years of experience, the most effective dosing regimen of ERT is still a subject of debate and varies from 15-30 U/kg/4 weeks vs 80 U/kg/4 weeks.

Methods. Adult Gaucher disease type I patients from two large European treatment centers, Amsterdam (AMC, N=49; median dose 15-30 U/kg/4 weeks) and Düsseldorf (HHU, N=57; median dose 80 U/kg/4 weeks) were included. Follow-up parameters included hemoglobin, platelet count, plasma chitotriosidase levels, liver and spleen dimensions, severe bone complications and scoring of bone marrow involvement by MRI of the femora. All parameters were matched at baseline and analyzed in two separate ways: comparison of baseline values vs values after one year and life table analysis (Kaplan-Meier). Results. There were no significant differences in genetic background, age, gender, number of splenectomies and SSI in any of the matched populations. Improvement in hemoglobin, platelet count and hepatosplenomegaly was not significantly different between both cohorts, whereas bone marrow involvement by MRI especially in patients with severe bone disease, and plasma chitotriosidase improved significantly faster in the higher-dosed group. Major bone complications rarely occurred in both groups. Conclusions. As improvement of hemoglobin, platelet count and liver and spleen volume is not dose-dependent, extensive organomegaly and cytopenia do not justify a high initial dose. The quicker response for bone marrow involvement upon a higher dose in severely affected patients is considered an important criterion to start a higher dose of enzyme. Chitotriosidase proves to be a sensitive indicator of dose effects and may be used in that respect to monitor response. The determination of the most cost-effective dosing regimen should be made individually and on the basis of a complete disease profile, including proper assessment of bone marrow involvement in addition to hematological, visceral and biochemical parameters.

Lung Resection for Invasive Pulmonary Aspergillosis in Neutropenic Patients with Hematologic Malignancies: Long-Term Results in Thirty Cases

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Invasive pulmonary aspergillosis (IPA) is a major cause of morbidity and mortality in neutropenic patients. Nevertheless, recent studies suggest that the outcome of IPA is improving due to early diagnosis (CT scan, antigenemia), use of new antifungal agents (Azoles and Echinocandins), and possibly in some cases to early surgical resection. We here report a retrospective one center study of 30 cases of IPA treated by surgical treatment from 1988 to 2005. Patients were 18 men and 12 women, with a median age of 50 years (15 - 74). The underlying diseases were AML, ALL, aggressive lymphoma and myeloma in 22, 5, 2 and 1 cases, respectively. Surgery was planned after hematologic recovery from the last course of chemotherapy during which IPA was diagnosed, either possible in 15 cases, probable in 14 cases or proven in 1 case (Ascioglio, CID 2002). Surgery consisted in 1 pneumectomy, 4 bilobectomies, 17 lobectomies, 6 wedge resections and 2 lobectomies with wedge resections. No perioperative deaths occurred and the median duration of hospitalisation was 12 days. Four patients presented post surgical complications (pneumothorax, pneumopathy, section of phrenical nerve and bleeding). The diagnosis of definite IPA was confirmed in all 30 cases. Immediately after surgery, 24 patients were able to receive subsequent intensive chemotherapy courses, including 11 stem cell transplant (SCT), either auto (4) or allogenic (7). In all cases, patients subsequently received postoperative antifungal therapy. During these new intensive chemotherapies, recurrent aspergillosis was observed in only 2 cases, (inducing 1 death from brain localization). Overall, with a median follow-up of 8.8 years (1-18), 86% of the patients are alive and the main cause of mortality was relapse, but not IPA. In conclusion, early surgical resection together with antifungal therapy allows definite diagnosis of IPA, and thereby IPA recurrence and early death due to hemovasculitis, and at last allows subsequent high-dose chemotherapy to treat the underlying hematologic disease.

The Role of DLL4 in Endothelial Progenitor Cell Function During Tumor Angiogenesis

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Backgrounds. In the adult, during tumour growth or in situations of vascular stress, angiogenesis is achieved not only by the recruitment of endothelial cells from neighboring blood vessels, but also by the mobilization of bone marrow (BM)-derived endothelial progenitor cells (EPCs). The differentiation and consequent incorporation of these circulating progenitor cells into foci of neoangiogenesis appears to be essential for the adequate assembly and function of the blood vessels. The Notch signalling pathway has been involved in vascular network remodelling and in arterial/venous identity of blood vessels during embryonic development. Aims. However, the function of this pathway in tumour angiogenesis still remains unclear, and is the subject of the present study. Methods. In this work, we first analyzed the expression of Notch signaling components in EPCs, isolated from mouse BM and in vessels that grow into xenografted human lymphoma. Results. Mouse EPCs expressed the receptor Notch1 and the ligands Delta-like 4, Delta-like 1 and Jagged 1. In situ hybridization analysis of the lymphoma xenograft showed that DLL4 expression level was higher and more frequently detected in the vessels than Notch1 and Notch4. Moreover, there was no arterial or venous restriction of the DLL4 expressing vessels within the
lymphoma xenograft. To evaluate in particular the role of Notch ligand DL4 in EPCs function, we investigated the in vitro differentiation potential, cell migration and vessel network formation of EPCs isolated from DL4+ mutant mice. Conclusions. These results obtained thus suggest that the Notch signalling pathway might play a role in EPCs function, mediating the crosstalk between EPCs and endothelial cells during tumour angiogenesis.

0884
PRESENT TRENDS IN THE MANAGEMENT OF INVASIVE Fungal INFECTIONS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES
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Background. Fungal infections are an important cause of morbidity and mortality in patients with hematological malignancies. Historically, treatment has been with amphotericin B and later its lipid formulations. However, new therapeutic agents have recently been introduced. The empirical use of antifungal therapy is today a standard approach in patients with persistent febrile neutropenia. However, the low incidence of invasive fungal infections (IFI) and the progress in the diagnoses and treatment of IFI have made the routine use of empirical antifungal therapy questionable. Aim. With the aim to include the present trends in the use of antifungal agents for the treatment of IFI we prospectively observed type, safety and efficacy of given antifungal treatment in patients with hematological malignancies during a recent 18-month period. At the same time we analyzed the impact of restricted use of empirical antifungal therapy on IFI related mortality. Patients and Methods. Data was collected from patients admitted for treatment of febrile neutropenia to our department from November 2003 through April 2005. All patients who had received antifungal therapy empirically or for treatment of IFI were included. Local guidelines recommended a restricted use of empirical antifungal therapy to patients with persistent febrile neutropenia (5 days or more), who were, after individual assessment, considered to be at high risk for IFI. Caspofungin was recommended for this indication. Voriconazole was recommended as primary therapy of invasive aspergillosis, while caspofungin in this setting was recommended as salvage therapy. Results. A total of 279 episodes of neutropenia and fever following chemotherapy were recorded. All patients were treated for hematological malignancies, predominantly acute leukemia (50%). Treatment of IFI was given during the management of 41 (14%) episodes of febrile neutropenia occurring in 35 patients (Table). Voriconazole (27 episodes) and caspofungin (14 episodes) were the only antifungal agents used as initial therapy. Two patients received the combination of caspofungin and voriconazole as salvage therapy. The rate of antifungal therapy success outcome was 76% (Table). Oral preparation of voriconazole was given from the first day of treatment to 88% of patients treated with this agent. In general, antifungal agents were well tolerated and only two patients had to discontinue treatment due to severe adverse event. The overall 4-week mortality rate was 8%. Two patients died from invasive pulmonary aspergillosis. Empirical antifungal therapy was given to 13 patients with persistent febrile neutropenia without any signs of local infection and resulted in successful outcome in 92% of cases. In 127 episodes of persistent febrile neutropenia antifungal therapy was deemed unnecessary and accordingly was not administered. In this subgroup of patients the overall 4-week mortality rate was 4% and 4 patients died of infection. No IFI related mortality occurred in this subgroup of patients. Conclusions. A better tolerability and efficacy of voriconazole and caspofungin together with the oral alternative of voriconazole have been lead to a shift in the use of antifungal agents for the treatment of IFI. A restricted use of empirical antifungal therapy was, in this setting, not associated with increased IFI related mortality.

Table 1.

<table>
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<th>Number of episodes</th>
<th>Successful outcome (%)</th>
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<td><strong>Empirical therapy</strong></td>
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0885
A SURVEY ON INVASIVE Fungal INFECTIONS IN SGT: PROPHYLAXIS, TREATMENT, INCIDENCE AND CLINICAL OUTCOME AMONG 660 PATIENTS TRANSPLANTED DURING 2000-2004
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Backgrounds. Historical incidence (80s and 90s) of IFI in recipients of SGT ranges from 10-25% with an overall case fatality rate of up to 70-90%. Aims. Here we report our findings regarding the demographics, microbiology, clinical outcome and risk factors for the development of IFI among patients who underwent SGT in 5 hospitals of Madrid (Spain). Methodology: A retrospective study of all the patients who underwent SGT in 5 units of Madrid (Spain) during 2000-04 was done. Results: 120 patients received alloSCT (18%), 56 from a sibling donor and 34 from alternative donors. In 59 cases (49%) a RIC regimen was employed. PB was the source of stem cells in 650 patients (98%). Lymphoma (27%), acute leukemia (182) and myeloma (160) were the main underlying diseases. 357 patients were in complete remission and 283 (45%) were transplanted for persistent disease in 42 patients (4%) had prior history of IFI. Determination of serum galactomannan was introduced in the last two years and data were available from 127 cases (20%). Nearly all patients received antifungal prophylaxis (639) (oral fluconazole in 576 cases−90%). An empiric antifungal treatment was instaurated in 190 cases (20%) and was more common in the allo population (40% vs 25%, p<0.007). Ambisome was the drug of choice in 120 cases (82%). IFI after SCT (EORTC criteria) occurred in 32 patients (4,8%) (possible: 17, probable: 7, proven: 8). Median day of diagnosis was day +277 (+275), and pulmonary disease was the most common clinical presentation. Aspergillus was the most frequently involved mold (60%). 16/82 patients with a diagnosis of IFI had died (50%), in 8/16 cases death was attributed directly to IFI and contributed in other 4 cases. IFI was more frequent among allo vs auto (4,4% vs 2,7%; p<0.001); AL vs other diseases (6,6% vs 5,7%; p=0.04); a previous history of IFI (17% vs 4%; p<0.02); severe GVHD (grades III-IV and extensive C-GVHD on IS treatment) (30% vs 2,7%, p<0.02) and disease not in CR (6,67 vs 3,47; p=0.059). A multivariate analysis selected type of transplant (allo) (p=0.003; RR: 2,76), previous IFI (p=0.04; RR: 3,9) and severe GVHD (p<0.04; RR: 4,02) as the main risk factors for the development of IFI. Conclusions. Our findings shows that IFI had a low impact on mortality in the present series, 9/660 (1,3%) and that current fatality rate among SCT with IFI was 28% (9/32) although the mortality in patients with IFI was 50%, higher that the non IFI cohort. Advances in clinical management, including anticipate diagnoses, a more appropriate use of antifungal drugs, and the presence of low numbers of really high-risk patients could be argued for explanation.

0886
RANDOMIZED TRIAL OF PREVENTION OF CATHETER-RELATED BLOODSTREAM INFECTION IN PATIENTS WITH HEMATOLOGIC AND ONCOLOGIC DISEASE
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Centre National de Greffe de Moelle Osse, Tunis, Tunisia

Backgrounds. Data from the National Nosocomial Infection Surveillance system (United States) between January 1992 and February 1998 showed that Catheter-Related Bloodstream Infection (CRBI) is the third most frequent nosocomial infection and accounted for 14% of all nosocomial infections. CRBI may be caused by fibrin deposition associated with catheters. Interventions designed to decrease fibrin deposition have the potential to reduce CRBI. In a previous randomised study, we have shown that the use of continuous infusion of low-dose (100 IU/kg per day) unfractionated heparin (UFH) was a practical and economical approach to the prevention of CRBI in patients with hematologic-oncological disease. Aim: The purpose of this study was to evaluate the role of heparin-coated central venous catheter (CVC) in preventing CRBI in patients with hematoma-oncological disease. Methods. This study was a randomised controlled trial in which patients were randomly assigned to receive either a heparin-coated CVC without a continuous infusion of low-dose UFH (heparin-coated group) or a non-coated CVC with a continuous infusion of low-dose UFH (control group). CRBI was defined
according to the difference in time to positivity.3 Results. Between April 2005 and February 2006, one hundred and twenty patients were randomly assigned. Two patients were excluded after assignment. Ultimately, 118 patients were analysed. CRBl occurred in 5% (3 of 59 catheters) of those in the heparin-coated group (2.2 events per 1000 days) and in 8.5% (5 of 59 catheters) of those in the control group (2.9 events per 1000 days) (p=0.7). Two and three patients experienced severe bleeding in the heparin-coated and control groups, respectively (p=0.5). We did not observe heparin-induced thrombocytopoiesis. Conclusion: The use of heparin-coated catheter is a safe and effective approach to the prevention of CRBl in patients with hematologic and oncologic disease.

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mas: breast, esophagus, colon, as well as on plasma of patients with thyroid and kidney cancers, multiple myeloma, malignant lymphoma. Aims. The aim of this investigation was to examine: (a) whether 31P NMR spectra of phospholipid extracts from plasma, PBMC, and BMMC are suitable for the analysis of phospholipid metabolism of blast cells from patients with AL, (b) whether obtained spectra allow to differentiate lymphoblastic acute leukemia (ALL) from myeloblastic acute leukemia (AML). Methods. 31P MRS spectra were obtained from phospholipids extracts of plasma (21 healthy volunteers, 44 patients with AL), PBMC (11 healthy volunteers, 52 patients with AL), and BMMC (38 patients with AL). Cellular phospholipids were isolated from mononuclear cells (MC) by means of Ficol buffy coat centrifugation. Methanol-chloroform precipitation of phospholipids extraction from 60°/6° C was performed according to the modified Folch’s method. 31P MRS analyses were conducted on AMX 300 Bruker spectrometer 7.05 T. Results. 31P MRS spectrum of phospholipid extracts from normal human PBMC consisted of 8 peaks due to following phospholipids: phosphatidylcholine (PC), phosphatidylcholine plasmalogon (CPLAS), lysophosphatidylcholine (LPC), sphingomyelin (SM), phosphatidylethanolamine (PE), phosphatidylinositol (PI), the spectrum of phospholipid extracts from normal human PBMC consisted of 8 peaks due to following phospholipids: phosphatidylcholine (PC), phosphatidylcholine plasmalogon (CPLAS), lysophosphatidylcholine (LPC), sphingomyelin (SM), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), cardiolipin (CL), and one due to MDPA. The peak due to LPC appeared only occasionally and not all spectra contained peak due to CL. However, the spectrum of phospholipid extracts from plasma consisted of 6 peaks due to phospholipids: PC, CPLAS, LPC, SM, PE, PI, and one due to MDPA. We observed, that spectra from phospholipid extracts from plasma and PBMC of patients with AL differed statistically from phospholipids of plasma and PBMC of healthy volunteers. Spectra obtained from phospholipid extracts of PBMC and BMMC patients with AL didn't differ. Spectra obtained from PBMC and BMMC of ALL patients differed significantly from AML patients. However, we didn’t observe any statistically significant difference within spectra from plasma (Table 1).

Table 1. Concentration of phospholipids.

<table>
<thead>
<tr>
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<th>ALL (nmol/l)</th>
<th>AML (nmol/l)</th>
<th>ALL (nmol/l)</th>
<th>AML (nmol/l)</th>
<th>ALL (nmol/l)</th>
<th>AML (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>2.01±1.58</td>
<td>1.62±1.08</td>
<td>0.32±0.18</td>
<td>0.40±0.18</td>
<td>0.24±0.16</td>
<td>0.42±0.26</td>
</tr>
<tr>
<td>CPLAS</td>
<td>0.05±0.08</td>
<td>0.06±0.06</td>
<td>0.02±0.03</td>
<td>0.05±0.05</td>
<td>0.01±0.02</td>
<td>0.05±0.05</td>
</tr>
<tr>
<td>LPC</td>
<td>0.08±0.11</td>
<td>0.07±0.09</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SM</td>
<td>0.50±0.43</td>
<td>0.42±0.32</td>
<td>0.04±0.05</td>
<td>0.09±0.06</td>
<td>0.02±0.04</td>
<td>0.11±0.09</td>
</tr>
<tr>
<td>PE</td>
<td>0.08±0.12</td>
<td>0.08±0.09</td>
<td>0.15±0.09</td>
<td>0.28±0.17</td>
<td>0.15±0.13</td>
<td>0.30±0.19</td>
</tr>
<tr>
<td>PS</td>
<td>-</td>
<td>-</td>
<td>0.01±0.02</td>
<td>0.04±0.04</td>
<td>0.01±0.02</td>
<td>0.04±0.04</td>
</tr>
<tr>
<td>CL</td>
<td>-</td>
<td>0.00±0.00</td>
<td>0.00±0.03</td>
<td>0.00±0.01</td>
<td>0.00±0.00</td>
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Conclusion. Our data show that 31P MR spectra from PBMC and BMMC are identical, both in position of peaks and concentrations of phospholipids. It may indicate, that blast cells from PB demonstrate the same metabolism of phospholipids as blast cells from BM. Only in PBMC and BMMC of ALL patients differed significantly from AML patients. However, we didn’t observe any statistically significant difference within spectra from plasma (Table 1).

0890
SAFETY OF A WEEKLY ADMINISTRATION OF 7.5 MG/KG OF LIPOSOMAL AMPHOTERICIN B FOR ANTIFUNGAL PROPHYLAXIS IN PATIENTS RECEIVING HIGH DOSE CONTIGUOUS RISK FACTORS FOR ACUTE GVHD AFTER REDUCED INTENSITY CONDITIONING ALLOGENEIC STEM CELL TRANSPLANTATION

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RIC regimens are increasingly used for allo-SCT in elderly or patients not eligible for standard myeloablative allo-SCT. Such regimens have yielded promising results in terms of decreasing early transplant-related toxicities. However, acute GVHD remains a matter of concern in this setting. Of note, the use of high dose CS for GVHD treatment increases the risk of severe fungal infections in this high risk population usually presenting several comorbidities. Therefore, prophylactic strategies aiming to reduce this risk are needed. We conducted a pilot single centre study in 15 adult patients receiving high dose CS (2 mg/Kg/day) for acute GVHD therapy after RIC allo-SCT. Treatment consisted of a 2 hour weekly infusion of 7.5 mg/kg LAB with a maximum of 8 total doses. The primary endpoint was the incidence of serious adverse events occurring during the course of prophylaxis treatment. Of note, safety was monitored with particular attention to nephrotoxicity in this relatively elderly population receiving concomitant nephrotoxic drugs such as cyclosporin A. Median age of these 15 patients with various hematological and non-hematological malignancies was 54 years (range, 40-70). Patients received a median of 4 weekly doses of LAB (range, 1-8), with 8 patients (53%) receiving at least 4 consecutive weekly doses. In terms of toxicity, 6 patients (40%) didn’t experience any sign of toxicity. One patient experienced a violent chest pain with transient extra-systoles, during the first infusion of LAB, and did not receive any subsequent infusions. Other mild and transient infusion-related reactions (fever, flush, tachycardia, orthostatic hypotension, pruritus, bone pain, abdominal pain) were observed in 5 patients, usually at time of first LAB infusion. Despite concomitant administration of cyclosporin A in all 15 patients, only 4 patients (27%) had to interrupt the course of prophylactic LAB (respectively after 8 (n=2), 4 and 7 infusions) because of renal toxicity (increase of serum creatinine ≥1.5 times from baseline values). Although long term efficacy of such antifungal prophylactic strategy is yet to be established, the results of this feasibility study demonstrate that a weekly dose of 7.5 mg/kg of LAB is relatively safe and well tolerated when given as prophylaxis in high-risk immuno-compromised patients receiving high dose CS for GVHD after RIC allo-SCT, despite concomitant administration of multiple nephrotoxic drugs.

0891
IMMUNITY AGAINST POLIO, DIPHTHERIA AND TETANUS AFTER CONVENTIONAL CHEMOTHERAPY TREATMENT FOR AML AND HIGH-GRADE LYMPHOMA

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Background. In a recent paper, subprotective antibody levels against diphtheria and tetanus were found in a majority of pediatric patients after treatment for high-risk acute lymphatic leukemia (Ök T et al. 2005). Information is scarce regarding immune reconstitution in adult patients who have undergone chemotherapy treatment for hematological malignancies. Aims. The aim of this study was to investigate whether protective antibody levels against diphtheria, tetanus and polio are retained in adults after intense chemotherapy treatment of acute myelogenous leukemia (AML) and high-grade non-Hodgkin’s lymphoma (HGNHL). Patients and Methods. Thirty-two patients, 18 males and 14 females, median age 61 (19-79) years, all in CR1 for a duration of >6 months after conventional treatment for AML (n=16) or HGNHL (n=16), were included. Twenty-nine healthy sex-matched persons, median age 60 (24-69) years, were enrolled as a control group. Immunity against polio types 1, 2 and 3 were assessed utilizing a standard neutralization assay, whereas antibody levels against tetanus and diphtheria toxoids were determined using an ELISA and a neutralization test, respectively. The minimum protective thresholds, as defined for clinical samples by the microbiology laboratory, were used to categorize patients and controls as immune or susceptible to infection. Results. Subprotective antibody levels against at least one of three polio serotypes were found in 10 out of 32 patients (31%), to be compared with 2/25 (8%) in healthy controls (p<0.05, χ2 test). Twelve out of 32 (58%) patients versus 4/29 controls lacked immunity against diphtheria (p<0.05). With respect to immunity against tetanus, the difference between patients and controls (4/32 vs 1/29 pts) was not significant (p=0.36). Summary. We report a high prevalence of subprotective antibody levels against polio and diphtheria in AML and lymphoma patients who were in CR after conventional therapy not encompassing stem cell transplantation. Provided that these results can be confirmed in a larger study, assessment of immunity status, and possibly vaccination, may be considered not only in patients with leukemia and lymphoma but also in other patient groups receiving intensive chemotherapy.
FLUOROCHINOLONE-RESISTANT ESCHERICHIA COLI IS THE MOST FREQUENT PATHOGEN ISOLATED FROM PATIENTS WITH HEMATOLOGIC MALIGNANCIES. RESULTS OF A PROSPECTIVE STUDY ON 364 CONSECUTIVE EPISODES OF FEVER AT A SINGLE INSTITUTION

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Background. Regular monitoring of the bacterial epidemiology at hematologic units has been recommended in order to evaluate the effects of the prophylactic and empiric antibacterial strategies adopted. Aims: We describe the most frequent pathogens involved in infectious complications and the emerging resistance to antibiotics. Methods. We analysed all the consecutive febrile/infectious episodes occurring to the 823 patients admitted to our Institution (248 Acute Leukemia, 253 Lymphoma, 195 Myeloma, 26 Myelodysplastic Syndrome, 28 Chronic Lymphocytic Leukemia, 10 Myeloproliferative Syndrome and 83 non neoplastic haematological patients) from June ‘04 to September ‘05. All the patients with expected neutropenia lasting for more than 7 days received prophylaxis with levofloxacin 500 mg/day. Results. Three hundred and sixty-four cases developed fever/infection (44.2% of all admission) and in 183 (49.6%) of these patients Gram- infection was clinically documented (bacteremia 46%, pneumonia 42%, urinary tract infections 7%, others 5%). One hundred and sixty-four of these patients were isolated in 157 microbiologically documented infections (37.6%) including 82 Gram- bacteria (50%), 66 Gram+ bacteria (40.2%), 14 fungi (8.5%) and 2 miscellaneous (1.5%). E. coli, Enterobacteriaceae other than E. coli, and Pseudomonas spp were the most frequently isolated bacteria-strains (respectively 25.2%, 9.8% and 10.4% of all isolates). Among Gram-, S. aureus, Coagulase-negative Staphylococci (CoNS) and Enterococci were the most frequent pathogens, accounting respectively 15.2%, 11% and 7.9% of all isolates. E. coli was statistically more frequent in patients affected by acute leukaemia (26/69, 38% vs 10/62, 16%, p<0.01), neutropenia <500/mm3 (31/81, 38% vs 7/56, 13%, p<0.01) and on prophylaxis with levofloxacin (26/66, 42% vs 8/43, 19%, p<0.05) S. aureus was associated with a non controlled underlying disease (24/91, 26% vs 1/46, 2%, p<0.01), neutrophil count >500/mm3 (20/56, 36% vs 5/81, 6%, p<0.01) and absence of prophylaxis with levofloxacin (11/45, 26% vs 3/66, 5%, p<0.01). Presence of central venus catheter (CVC) did not significantly predispose to CoNS infections (16/98, 16% vs 2/39, 6%, p=NS). Gram- bacteria showed resistance to Fluoroquinolones (Fq) in 42/61 cases (68.9%) associated by multivariate analysis only to prophylaxis with levofloxacin (OR 9.85, IC 2.52-42.01, p=0.002). Specifically Fq-R resistant E. coli represented 91.7% of E. coli isolates (33/36). Hence it represented the pathogen most frequently isolated (21.3% of all isolates) among hematopoietic patients admitted to our Institution. Sixteen of the 25 (64%) Staphilococcus spp showed resistance to methicillin (MR), which was associated by multivariate analysis to prophylaxis with levofloxacin (OR 15.68, CI 1.05-252.13, p=0.047) and CVC (OR 20.23, 1.34-208.69, p=0.006)and not to PTAL association. Conclusions. In contrast with the recently reported prevalence of Gram+ bacteria in most haematological units, a shift toward Gram- bacteria, particularly Fq-R E.coli, was observed in our Institution. The role of levofloxacin prophylaxis in changing the epidemiological pattern and inducing Fq-R and MR needs further investigation.

CIRCULATING ENDOTHELIAL PROGENITOR CELLS IN PATIENTS WITH ANCA-ASSOCIATED VASCUITIS

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Introduction. There are two essential types of endothelial cells circulating in blood. First type are mature endothelial cells (CECs), which numbers are increased in microcirculation disorders such as ANCA associated small vessels vasculitis (AAV). These cells are connected with vascular damage. The other type are circulating endothelial progenitor cells (EPCs), which originate from bone marrow and play a crucial role in vasculogenesis, angiogenesis and repair. Aims. The study goal was to compare the frequency of immature EPCs in these patients. Methods. Circulating EPC numbers were determined in 35 patients with AAV, including 16 patients with newly diagnosed active disease without prior immunosuppressive treatment, 15 patients with active disease already treated by immunosuppressive therapy (pulse i.v. cyclophosphamide and periarticular corticosteroids) and 10 patients in remission of the disease. Six patients were investigated twice (at diagnosis and in remission). We have used three groups of controls: 15 patients with non-AAV renal damage (patients on long-term hemodialysis), 9 patients suffering from macrocirculation disorders (ischemic disease of lower extremities) and 25 healthy volunteers. EPCs were enumerated by colony forming unit assay. 15-20ml of peripheral blood was centrifuged on Ficol-Hypaque gradient (Pharmacia, Upssala, Sweden) and mononuclear fraction was cultivated in the EndoCultTM medium (StemCell Technologies, Vancouver, Canada) according to manufacturer instructions. Results. The number of EPC was significantly lower in patients with AAV compared to healthy volunteers (median 0.5 vs 12.3 EPC-CFU/ml blood, p<0.001). We did not find any statistical difference in numbers of EPC among groups of AAV patients before and after beginning of treatment and a group of patients in remission on maintenance immunosuppression. We have also found no correlation between the number of EPC and the Birmingham Vasculitis Activity Score (BVAS), level of C-reactive protein, plasma creatinine or titre of ANCA. Patients with ANCA anti-PR3 antibodies had a trend toward lower numbers of EPC compared to those with anti-MPO antibodies (median 0.18 vs 5.15 EPC-CFU/ml blood, p=0.08). The number of EPC in patients on long-term hemodialysis was also significantly lower than in healthy volunteers (median 1.9 vs 12.3 EPC-CFU/ml blood, p=0.001) and not statistically different from the number of EPCs found in patients with AAV. Patients with macrovascular disorders had non-significantly lower numbers of EPCs compared to healthy volunteers and significantly higher than patients with AAV (6.18 v. 0.5, p=0.035). CONCLUSION: Higher numbers of mature circulating endothelial cells, numbers of circulating endothelial precursors are significantly lower in patients with ANCA positive vasculitis. This may reflect serious endothelial damage on one hand, coupled with diminished ability of endothelial healing. Immunosuppressive treatment, which is frequently also cytotoxic, may suppress not only the microvascular inflammation but also the endothelial healing process. Low numbers of EPCs in patients with terminal kidney disease may reflect the accelerated atherosclerosis found in uremia.

0893
SIGNIFICANCE OF ENDOTHELIAL MICROPARTICLES, PLATELETS, AND LEUKOCYTE ACTIVATION IN PATIENTS WITH ACUTE CORONARY SYNDROME

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Backgrounds. The details of interactions between endothelium, platelets, and leukocytes in ACS are not well understood. Aims. The purpose of this research was to determine the levels of platelet, leukocyte, and endothelial activation and markers of cellular interactions in patients with acute coronary syndrome (ACS). Methods. We studied 55 patients with VTE and compared 55 healthy controls. We used flow cytometry to measure: 1) endothelial microparticles (EMP) identified by CD144 and EMP(31) or EMP(11) or EMP(31); 2) platelet-leukocyte complexes (CD31/CD42b); 3) surface expression of P-selectin in platelets and CD11b in leukocytes; 4) EMP-monoocyte conjugates (percentage of monocytes positive for E-selectin); and 5) platelet-leukocyte conjugates (PLC) expressed as percentage of leukocytes positive for CD41. Results.

Patients with ACS had marked elevations of EMP(31) (2.213 vs. 372 counts/microl, p<0.01), EMP(31)/CD42b (579 vs. 251 counts/microl, p<0.001), and EMP-monoocyte conjugates (3.2% vs. 2.3%; p<0.01), as well as increased activation of platelets (31.9 vs. 4.8 fluorescence intensity units for P-selectin; p<0.001) and leukocytes (12.5 vs. 6.9 U for CD11b; p = 0.01). Elevated in ACS were PLC (59.6% vs. 37.4%; p<0.01). Expression of CD11b in leukocytes strongly correlated with PLC (r = 0.682, p<0.001). Conclusion. Marked activation of platelets, leukocytes, and endothelial cells occurs in ACS, which involves the release of EMP and formation of EMP-monoocyte conjugates and PLC. These findings support prior studies suggesting that release of EMP and their binding to monocytes are key events in thrombogenesis. Our findings also support the concept that the formation of PLC regulates leukocyte activation and participates in linking thrombosis with inflammation.
A COMPARATIVE STUDY OF RESPONSE TO EMPIRIC AMPHOTERICIN B DEOXYPHOSPHOCHOLATE ON DAY 4 OR DAY 8 OF FEBRILE NEUTROPENIA

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Background. Febrile neutropenic patients are at greater risk of getting bacterial and fungal infections. Initial therapy for these patients consists of broad spectrum antibiotics. Persistent fever without localisation in spite of more than 3 days of broad spectrum antibiotics including vancomycin qualifies for initiation of empiric antifungal therapy. However, the timing for empiric antifungal therapy can vary from 3 days to 8 days of non response to antibiotics. Aims. We choose to determine the response of empiric amphotericin B deoxycholate starting either on day 4 or day 8 in febrile neutropenic patients not responding to broad spectrum antibiotics and without localisation of fever. The study also examined the side effects related to amphotericin B deoxycholate in this group of patients. Methods. Fifty six neutropenic patients with persistent fever despite 72 hours of antibacterial therapy were randomly assigned to receive amphotericin B either starting from day 4 (group A, n=27) or starting from day 6 (group B, n=29). Patients in both the groups were evaluated for efficacy and safety of study drug by the clinical criteria, frequent cultures, radiological procedures and laboratory parameters. A response was defined as satisfactory at the end of therapy if the patient was afebrile for 48 hours, had absolute neutrophil count (ANC) > 0.5×10^9/L, and did not require study termination due to patient’s withdrawal from the study, drug toxicity, and persistent fever requiring change in therapy or death due to any cause. Results. The median age of patients in group A and in group B was comparable (23 versus 25 years). There were 17 males in group A and 18 males in group B. The patient population consisting of acute myeloid leukemia, acute lymphoblastic leukemia, aplastic anemia, non Hodgkin lymphoma, Hodgkin disease, chronic myeloid leukemia and multiple myeloma was equally distributed in two groups. A satisfactory response occurred in 82.2% of patients in group A and 69.0% of patients in group B (p=0.209). Time taken for resolution fever was considerably less in group A as compared to group B (5.4±3.9 days versus 11.3±4.0 days, p=0.001). It was also reflected in total dose requirements between groups A & B respectively (529±258.4 mg versus 790±750.3 mg, p=0.028). The factors (age, sex, body mass index, baseline temperature, diagnosis, ANC) affecting the satisfactory response rate to amphotericin B were not statistically significant in two groups. Documented fungal infections were seen in 4 patients (14.8%) in group A as compared to 11 patients (37.9%) in group B (p=0.072). The adverse side effects of amphotericin B (nephrotoxicity, hypokalemia, hypomagnesaemia) occurred at similar rates in the two groups. None of the risk factors studied (age, sex, body mass index, total dose of amphotericin B, baseline renal functions or exposure to various nephrotoxic antibacterial antibiotics) could be implicated in the causation of nephrotoxicity in group B. Conclusions. We conclude that initiating early empiric (day 4)amphotericin B deoxycholate in persistent febrile neutropenic patients leads to early response rate and decreased dose requirements of amphotericin B without increased risk of nephrotoxicity.

THE NEUROPATHIC SPECTRUM OF GAUCHER DISEASE: A SINGLE-CENTRE CLINICAL EXPERIENCE WITH 15 PAEDIATRIC PATIENTS

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Backgrounds. Gaucher disease patients are deficient in activity of the lysosomal enzyme glucocerebrosidase. As a consequence, accumulation of glucocerebroside occurs in macrophages in tissues throughout the body. An infectious disease manifests develop in part as a consequence of secondary damage. In neuropathic Gaucher disease, the central nervous system is also affected. Aims. We studied the clinical characteristics observed in a cohort of paediatric chronic neuropathic Gaucher patients under our care in order to delineate the spectrum of systemic and neurological presentations. The data were compared with clinical data collected from another cohort of 20 paediatric patients with non-neuropathic (type 1) Gaucher disease. Methods. All 15 neuropathic patients were thoroughly assessed at an initial evaluation (detailed history, physical examination, assessment of developmental status, and measurement of haemoglobin, platelet count and biochemical disease markers). Spleen and liver volumes were assessed (sonography) and chest X-ray, plain X-ray of the pelvis and DEXA scanning of the distal ulna were performed. Patients underwent comprehensive neurological examination, including evaluation of strabismus, eye movements and brain auditory evoked potentials (BAEP). Results. Compared to non-neuropathic patients, chronic neuropathic patients had significantly earlier diagnosis, significantly greater anaemia, and higher serum chitotriosidase activity, as well as ACE and lysozyme levels. Most of our patients with severe neurological involvement had pronounced splenomegaly in conjunction with bone and lung manifestations. A markedly higher degree of radiological evidence of pulmonary interstitial involvement and higher frequency of skeletal complaints were apparent. A considerable variety in types and combinations of neurological symptoms was observed. Prominent neurological abnormalities with early development of a saccade initiation failure was ubiquitous in our series and combined with strabismus and saccade initiation failure was common. BAEP abnormalities were also common. A large percentage of our patients had severe neurological disease with hypertelorax and ataxia and multifocal and/or myoclonic epileptic manifestations. Strabism as the first clinical neurological manifestation were detected in approximately half of our patients. Summary/Conclusions. The majority of our neuropathic Gaucher patients first had systemic manifestations of the bone disease and subsequently developed neurological abnormalities as the first neurological symptom. Therefore, we recommend that objective eye movement assessment is carried out in all children diagnosed with Gaucher disease. Presentation and progression of neuropathic disease was remarkably variable and, in fact, each patient is unique. Although the rigid historical categorisation of neuropathic Gaucher disease variants is still used by many, we are of the opinion that it fails to express the variability. Systemic disease presentations in chronic neuropathic Gaucher represented a more severe, early progressive condition than in type 1 Gaucher.

CAUSES OF INCIDENTAL NEUTROPENIA IN ADULTHOOD

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Backgrounds. The incidental discovery of neutropenia during routine blood counting represents a common problem for clinicians. However, there are no reported data of systematic evaluations of adults with incidental neutropenia. Aims. We aimed to identify the causes of incidental neutropenia in adults. Methods. Ninety-seven adults with incidental neutropenia were submitted to a clinical and laboratory approach, including complete and serial blood counts, direct and indirect antiglobulin test, bone marrow smear and biopsy, assessment of folate, vitamin B12 and iron status, serum liver enzymes, serum proteins, serological exams for hepatitis B and C virus, cytomegalovirus, human immunodeficiency virus and toxoplasmosis, detection of anti-nuclear and anti-DNA antibodies and rheumatoid factor, dosage of free thyroxin and thyrotropin, chest roentgenogram, and abdominal ichnography. The diagnosis of neutropenia due to exposure to chemical agents and hypocalcemia was verified by the granulocytic lineage in the bone marrow. The infectious, autoimmune, haematological and thyroid diseases, and nutritional deficiency-related neutropenia were defined when diagnoses of the diseases or condition were established by specific laboratory exams. The recovery of the neutrophil count with the resolution or control of the disease was also required for the identification of the causes. Ethnologic neutropenia was defined in individuals of African ancestry, in whom neutropenia was also present in other relatives. Drug-related neutropenia was diagnosed in patients under treatment with drugs, in whom the neutrophil count reached the normal value when the treatment withdrawn. Cyclic neutropenia was used to our non-neuropathic patients, chronic neuropathic Gaucher disease and the diagnosis of chronic idiopathic neutropenia of the adult (CINA) was established when none of the above mentioned causes was found. Results. CINA was identified in 34.0% of the individuals, neutropenia due to exposure to chemical agents in 16.5%, infectious diseases in 9.3%, autoimmune diseases in 9.3%, haematological diseases in 9.3%, thyroid disorders in 8.2%, ethnic neutropenia in 7.2%, drug-related neutropenia in 2.1%, cyclic neutropenia in 2.1%, and iron deficiency in 2.1%. Recovery or improvement of the neutrophil count was seen upon treatment or recuperation from infectious, autoimmune, haematological and thyroid diseases, and iron supplementation. Conclusions. We conclude that the evaluation of individuals with incidental neutropenia using haematologica/the hematology journal | 2006; 91(s1) | 329
a structured approach may possibly identify the identification of clinically silent diseases, and provide the opportunity for early treatment, avoiding complications of the diseases and consequences of neutropenia.

**0898**

**LONG TERM FOLLOW-UP OF TYPE 1 GAUCHER DISEASE PATIENTS. A RETROSPECTIVE ANALYSIS**


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**Background.** Gaucher disease (GD) is a rare familial disorder. It is caused by mutations in the glucocerebrosidase gene that causes a deficiency in β-glucocerebrosidase activity. In result, cleavage of glucose from ceramide is impaired and glucose-ceramide accumulates within cells. Three clinical phenotypes are recognized. Type 1 is the non-neu-

**Conclusions.** In respect to ERT, this study confirms the lit-

**0899**

**THE SAFETY AND EFFICACY OF CONTINUOUS DYNAMIC DOSE ANTICOAGULATION DURING CHEMOTHERAPY-INDUCED THROMBOCYTOPENIA**

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**Background.** The optimal management of patients with haematologi-

**0900**

**SURVIVAL IN MULTIPLE MYELOMA PATIENTS IS NOT AFFECTED BY DEVELOPMENT OR RECURRENTENCE OF THROMBOEMBOLISM**

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**Backgrounds.** Patients with cancer who developed venous thrombo-

**Results.** The exam-

**Methods.** The total GD Type 1 patients (14) diagnosed at our outpatient department was enrolled to this retrospective analysis. All but 2 patients have therefore been on ERT for at least 1 year and the longest follow-

**Summary.** The results of this study support the need for a structured approach to the identification of clinically silent diseases and provide the follow-up in order to enhance the understanding of this rare disease and to assess patients and their response to therapy over time, with the ultimate goal of improving the clinical outcomes through the definition of specific strategies.

**Conclusions.** The median number of days of thrombocytopenia <150×10⁹/L was 19 (range 9'40). Enoxaparin was delivered at full dose on 31% of thrombocytopenic days, at reduced dose on 63% of days, and withheld on 6% of days. Of the days where enoxaparin was withheld, 45% were the result of procedures; 20% bleeding; and 35% other reasons, including refractory thrombocytopenia <20×10⁹/L. Major bleeding rate occurred in 5.4% of all patients. In the prospective group, three patients experienced episodes of minor bleeding. No major bleeding was observed in this group. In the retrospective group, two patients developed major gastrointestinal bleeding requiring endoscopic intervention and transfusion while receiving reduced dose anticoagulation with platelet counts of 20×10⁹/L. One developed bleeding requiring surgical intervention at a wound site when the platelet count was 19×10⁹/L and enoxaparin had been withheld. Data regarding minor bleeding episodes were not available for patients in the retrospective group. No thromboembolic complications were identified in either group. **Conclusions.** The strategy employed here was not associated with excess bleeding. It was effective in preventing recurrence and exten-

**Methods.** Patients with newly diagnosed myeloma patients were enrolled in a study, which included induction phase with VAD, DCEP, CAD and DCEP followed by tandem high dose chemotherapy and peripheral blood stem cells (PBS) transplantations. Patients were randomly assigned upfront to receive Thalidomide or not. Both arms received identical chemotherapy. Patients were followed and clinically indicated underwent radiological studies to confirm a suspected VTE.
A total of 155 patients experienced VTE (median follow up of 47 months). Unbalanced baseline characteristics were balanced between patients experiencing VTE versus others who were female gender, more frequent in the non-VTE group on thalidomide, CRP > 8 mg/dl, and II<sup>6</sup> > 9 µg/mL were more frequently observed in the VTE group on no thalidomide. Median (63%) of the non-VTE group had significant differences in prognostic factors for survival (chromosomal abnormalities, albumin level, β2-microglobulin, CRP) were seen. OS of the entire group by VTE status is shown (Figure 1). VTE recurrence was modest and not significantly different in those who resume thalidomide. Conclusion: Our experience shows that a thromboembolic event during treatment for myeloma patients does not affect survival.

**0901**

**LOW-DOSE WARFARIN DECREASES THE INCIDENCE OF THALIDOMIDE ASSOCIATED VENOUS THROMBOEMBOLISM IN PATIENTS WITH MULTIPLE MYELOMA**

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Introduction. T and its analog lenalidomide have established their role as novel antineoplastic agents. VTE is a common and often problematic side effect of these agents. The incidence of VTE increases substantially (up to 26%) when T is combined with other therapeutic agents such as dexamethasone (D), anthracyclins (A) or platinum compounds. Different approaches for T associated VTE prophylaxis have been explored without any consensus to date. Since the underlying pathologic event remains unknown, the optimal method for prophylaxis is also undefined. We prospectively investigated the use of low-dose warfarin for the prophylaxis of VTE in MM pts treated with T containing regimen. Methods. All pts treated with T containing regimens at our institute receiving prophylaxis with low-dose warfarin were evaluable. Warfarin was given at doses 1 or 2 mg for actual body weight of < 70 kg or > 70 kg respectively, which is continued for the duration of the T treatment. Concurrent aspirin or other anticoagulants were not given. Pts who were fully anticoagulated for a prior VTE or other VTE risk factors are excluded from this analysis. Results. Eighty-two consecutive MM pts, median age 60 years (range 43-82) who received low-dose warfarin for T containing regimen are reported here. T was given in combination with either dexamethasone, VAD regimen, Bortezomib (B) or bortezomib/doxil. Of these 38 are male and 44 female. Median dose of T was 200 mg per day (range 50-300). Duration of therapy with T varied with the regimen used with a maximum duration of 6 months. Of these pts, 54 (63%) received T with D containing regimen, 56 (68%) with A and 31 (38%) with B containing regimen. Some pts received multiple T containing therapies during their clinical course. Four (4.8%) out of 82 pts were noted to have a VTE. Conclusion. Although we continued to see VTE with T, our experience suggests that low-dose warfarin can effectively decrease the overall incidence of VTE in pts treated with T containing regimens. Interestingly, none of the pts who received T with doxil experienced any episode of VTE. These findings warrant further investigation of low-dose warfarin as VTE prophylaxis for T based therapies.

**0902**

**PRESCRIPTION OF LOW-MOLECULAR WEIGHT HEPARIN FOR PROPHYLAXIS AGAINST VENOUS THROMBOSIS-EMBOLISM IN MEDICAL PATIENTS**

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Thromboprophylaxis is vital to avoid thrombotic complications which may account for over 25,000 deaths per year in hospital admissions in the UK annually. A previous audit of medical thromboprophylaxis in 2004 in our institution, a large teaching hospital, demonstrated a poor level (22%) of appropriate prescribing. Evidence based guidelines were developed, disseminated and implemented in early 2005. A reaudit of the appropriate prescribing of thromboprophylaxis was then undertaken to establish the proportion of medical patients at risk of VTE correctly prescribed LMWH for thromboprophylaxis. Data were collected through a retrospective audit of medical notes and prescription charts. All medical patients admitted under a consultant in a week in April 2005 were included as long as their length of stay was greater than 24 hours. The audit replicated the methodology employed in the previous audit and utilised an existing audit tool. 196 patients were included. The median age was 72 (range 18-96) years. 179 (91.5%) patients were aged 40 or over (26.5%) patients in the sample were female. As documented risk assessment was poor (10%) satisfactory risk assessment was defined as those patients in whom appropriate thromboprophylaxis in a timely manner was prescribed. 51 patients were excluded from analysis when the thromboprophylaxis exclusion criteria were applied. Of 145 valid cases thromboprophylaxis was indicated in 34 (23.4%). Of 54 patients regarded as appropriate to receive thromboprophylaxis 19 (56.5%) were prescribed it. The remaining 15 patients were not prescribed thromboprophylaxis and in 14 cases neither a risk assessment nor a reason for omission was documented in the medical notes. Thromboprophylaxis prescribing rate has increased from 22% (2004) to 56% (2005). This is encouraging, but further improvement is required. A Trust Thrombosis Committee has been formed and a quarterly hospital-wide audit and a new thromboprophylaxis nurse specialist post are planned.

**0903**

**CENTRAL VENOUS CATHETER-RELATED THROMBOSIS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES AND SCREENING WITH DOPPLER-ULTRASOUND**

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Backgrounds. Central venous catheters (CVCs) are vital component of care for patients with hematological malignancies. The use of CVCs provides an important means of venous access. CVCs are associated with the long-term risks of thrombosis and CVC-related thrombosis causes significant morbidity in the patients. However, not all of the thrombosis is symptomatic. Aim. To estimate the incidence of CVC-related thrombotic complications in hematological malignancies. Methods. We designed a prospective, observational study in our department. A total 78 CVCs in 42 consecutive patients with hematological malignancies were included in the study (males 28.6%, mean age 37.81 years ± 2.89). Most frequent diagnosis was acute leukemia (67%). Three lumens catheters were inserted in the subclavian vein in all of the patients. A record of all complications and catheters loss and final out come were analyzed. Blood samples for thrombophilia screening tests were taken from all patients before insertion of CVCs. All patients underwent serial Doppler-ultrasound until CVC removal and we evaluated whether clinically manifest thrombosis could be predicted by screening with Doppler-ultrasound. Patients were clinically assessed each day for signs and symptoms of thrombosis. In case of clinically suspected thrombosis, routine diagnostic and therapeutic procedures were done. Results. A total 78 catheters remained in subclavian vein for a median of 43 days (range 4-140). Total CVCs related thrombosis was observed in 6.4% (5/78) of patients. Of the 5 patients with thrombosis, 3 had superficial thrombosis by Doppler-ultrasound and no of them developed clinically manifest thrombosis later. Two patients had clinically manifest thrombosis without prior abnormal Doppler-ultrasound. Thrombosis of the catheters lumen (diagnosed upon the inability to aspirate blood with or without inability to flush occluded catheter) occurred in 20.5% (16/78). Catherter loss rate due to complication was 6.4% (5/78); 2 infection, 1 catheter thrombosis, 2 venous thrombosis). Neither total parental nutrition (p=0.46) nor difficult insertion of catheters (p=0.37) were related to thrombosis. Four patients had activated protein C-resistance (APC-R) and one of them had internal jugular vein thrombosis. Conclusion. The
incidence of clinically overt CVC-related thrombotic complications in patients with hematological malignancy is not negligible. The thrombosis of the lumen of the catheter is the frequent complication of central vein cannulation. However their necessity of catheter removal is negligible. Although symptomatic disease was not developed in our cases of subclinical thrombosis, doppler-ultrasound screening may be useful to identify the patients with subclinical thrombosis that require antithrombotic treatment.

**0904**

**DOPPLER ULTRASOUND ASSESSMENT OF SUBCLAVIAN VENOUS BLOOD FLOW AFTER THE IMPLANTATION OF A CENTRAL VEIN CATHERETER IN CHILDREN WITH MALIGNANCY**

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A central vein cannulation is a routine procedure in the management of children with cancer. This long lasting, permanent venous access, resulting in a significant improvement of the quality of life of these patients is usually achieved by the implantation of a tunneled central venous catheter (CVC) into one of the subclavian veins. The undisputable benefit of this procedure is limited by its side effects, necessitating careful lifelong monitoring of the clinical course. The complications of the procedure are uncomplicated however the late sequelae of subclavian veins catheterization remain obscure. *Aims of the study*. This study aimed to assess venous blood flow in subclavian veins of pediatric cancer patients in which CVC had been previously implanted. *Methods and Materials*. The study comprised 57 children (15 girls and 42 boys, aged 3-19 years, median 9 years) with pediatric cancer (ALL-27, ANL-L4, Hodgkin’s disease-5 and neuroblastoma-1 patient). In all of these children the central, tunneled catheter was inserted via the subclavian vein. The lumen of the CVC was rinsed once daily with heparin-lock (50 U/ml). The CVC was assessed using a Doppler ultrasound (ATL HDI 3500; 11 MHz linear head) after CVC removal. The time from CVC removal to Doppler examination ranged from 1 to 54 months (median 23 months). *Results*. None of studied patients had clinical symptoms of subclavian vein blood-flow disturbances. Abnormal blood flow in the subclavian vein was found in 22 of 37 patients (59,46%). These abnormalities included: 1) signs of subclavian vein stenosis with no signs of thrombosis - in 11 of 37 (29,73%) patients, 2) signs of venous thrombosis - in 4 of 37 (10.81%) patients, 3) increased pulsation amplitude and phase - in 7 of 37 (18.92%) patients. In the remaining 15 (40,54%) the subclavian vein blood flow was normal. The mean CVC life span in both groups of patients: with normal (n=15) and abnormal (n=22) subclavian vein blood flow did not differ significantly (275 vs 232; p=0.09). Conclusions. 1 A substantial proportion of children with uncomplicated course of central vein cannulation reveals the late ultrasound sequela of the procedure in form of subclavian vein stenosis and/or venous thrombosis. 2. The clinical relevance of these changes remains unknown and requires a long follow-up of larger groups of patients.

**0905**

**TIME COURSE OF INFLAMMATION AND PROTHROMBOTIC PARAMETERS IN ACUTE CORONARY SYNDROME**


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**Background**. The aim of this study is to assess the dynamics and magnitude of the thrombosis plasma markers in peripheral blood, and the relation to the degree of the inflammatory response across the spectrum of the acute coronary syndrome. Methods. Fifty patients with acute coronary syndrome; 10 with ST elevation myocardial infarction (STEMI), 10 patients with non-ST elevation myocardial infarction (NSTEMI), 10 patients with unstable angina (UA), 10 patients with stable angina (SA) and 10 comparable healthy controls were enrolled. The values of von-Willebrand factor antigen (vWF), thrombin-antithrombin complex (TAT) and prothrombin fragment 1+2 (F1+2) were assessed in peripheral blood using an enzyme linked immuno-assay method. The samples were collected on admission, daily during the first week, and days 14, 21 and 30 after the coronary event.

The inflammatory response was determined by the maximum levels of C-reactive protein for every patient at the same times. Kruskall-Wallis test and Spearman’s Rho test were used to establish relationship between these markers. Results. There were an increased prothrombotic response associated to myocardial damage, the values of vWF, TAT and F1+2 in patients with acute coronary syndrome were increased since the first day from the onset of symptoms, peaking on 4th or 5th day, and revert to normal levels after 1 month. There were significantly higher levels in patients with myocardial infarction related to the other diagnostics (< 0.01), specially at admission and at peaking plasma levels of these parameters (table 1). There were positive correlations between vWF & CPR (r=0.67), TAT & CPR (r=0.59) and F1+2 & CPR (r=0.78), VD=0.001. Conclusions. This study demonstrates a marked prothrombotic response across the spectrum of acute coronary syndrome with correlation to the myocardial damage. The inflammatory response is closely associated with the prothrombotic response.

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<th>Table 1. Median values at admission (a) and maximum level (b).</th>
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<td>STEMI</td>
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**0906**

**ELECTIVE SURGERY IN ANTICOAGULATED ATRIAL FIBRILLATION PATIENTS WITHOUT BRIDGING THERAPY WITH LMWH**

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**Backgrounds**. When oral anticoagulant therapy are discontinued for surgery, low-molecular weight heparin (LMWH) is often used as bridging therapy. However, this practice has never been evaluated in randomized clinical trials. In our Hospital, the bridging therapy with LMWH is only used in prostatic valves or embolism high risk atrial fibrillation patients without bridging therapy. *Methods*. Retrospective observational study, from January 2000 to March 2005. Oral anticoagulant therapy was stopped 4 days before the elective surgery in atrial fibrillation patients without previous cardioembolism episode. If INR was >1.5 on the day of surgery, perioperative heparin was considered. When it was possible, oral anticoagulant was restarted the evening of surgery. Venous thrombosis prophylaxis was performed, when necessary, with dalteparin (5000 IU daily) starting 12 hours before surgery and until the INR was >1.9. Medical records (including emergency attentions) were reviewed to check arterial embolism or bleeding episodes until 90 days after surgery. Results. 150 surgeries were eligible from 130 patients (73 men) with a median age of 76 years-old (44-91) and at least one of the following embolism risk factors: mitral valve disease, age >75, hypertension, diabetes mellitus or cardiac insufficiency. The majority procedures were ophthalmic surgeries (95), followed by orthopaedic, digestive system, urologic, gynecological and otolaryngology surgeries (31, 24, 20, 9 and 1 respectively). 74% of the procedures were ambulatory major surgery, 23% major and 3% minor surgery. No surgery was postponed. In 35% of cases venous thrombosis prophylaxis was made. One patient (0.5%; 95% CI, 0 to 1.1) had a transient ischemic attack 21 days after surgery and 2 patients (1.1%; 95% CI, 0.4 to 1.8) had an episode of bleeding ( one mild metrorrhagia after gynecological surgery and one acute cerebral hemorrhage 2 months after surgery). Conclusions. The arterial embolism ratio in our study is similar to the reported in anticoagulated patients when LMWH is used as bridging therapy. Oral anticoagulation treatment in atrial fibrillation patients without previous cardioembolism episode could be discontinued for surgical procedures without using LMWH bridging therapy. This strategy simplifies the perioperative management of anticoagulation in these patients, reduces the cost and avoids LMWH adverse effects without increasing the embolic risk.
0907  
VALIDATION OF THE COMPUTERIZED DECISION SUPPORT SOFTWARE TAOCHECK TO MONITOR ORAL ANTICOAGULANT THERAPY

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Backgrounds. Many clinical trials have demonstrated the utility of computer-based dosage programs to monitor oral anticoagulant therapy (OAT) in outpatients. However some of them are not already validated. Aims. We carried out a prospective, randomized trial to validate the efficacy of the computer application Taochek (Roche Diagnostics). Methods. From March to June of 2004, 118 outpatients on OAT in maintenance phase (more than 3 months under OAT) were randomized to two groups: 56 patients into the experimental group (Taochek-aided dosing) and 62 into the control group (experienced physician dosing). There were not differences between two groups regarding age, sex, and diagnosis to AOT. Patients did not know the allocation. Dosing recommendations made by Taochek could be overridden by a physician. The comparison between both groups was performed by individual proportion of time spent within the therapeutic target INR range and the INR comparison between both groups was performed by individual proportion of time spent within the therapeutic target INR range (p=0.014); Taochek group spent the 81.6% of time into INR±0.3 target range vs 94.4% of control group (p=0.599). The number of appointments every 30 days in the Taochek group was higher than in the control group (median 1.4 vs 0.9; p<0.001). From a total of 129 determinations in the Taochek group, 6 times (5%) the dose was not proposed by the software or was overridden by the physician and in 5 time (4%) the first day schedule dose was modified. Conclusions. Our study demonstrated that OAT can be provided at least as well by computerized decision support software Taochek as by experienced physician. However the Taochek-aided dosing group required more number of appointments per patient than the control group. Taochek software is useful for the AOT control and offers an effective help to inexperienced clinical staff.

0908  
RETROSPECTIVE REVIEW OF INDICATIONS AND RESULTS IN 4000 CONSECUTIVE D-DIMER SAMPLES

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D-dimers are used to assist diagnosis of venous thrombo-embolism (VTE), but are often inappropriately requested and interpreted. We retrospectively reviewed 4606 sequential d-dimer (DD) requests. We documented indication for request, repeat requests and compared results at different levels of raised DD. Our current DD test-kit is auto dimer (Trinity biotech), which has an upper limit of 180. Out of the 4606 requests, 2063 were ‘negative’ and 2603 were raised. The indications for DD requests were, in the large majority of cases, relating to diagnosis or exclusion of VTE. In a small minority of cases, the request related to diagnosis to AOT. Patients did not know the allocation. Dosing recommendations made by Taochek could be overridden by a physician. The comparison between both groups was performed by individual proportion of time spent within the therapeutic target INR range and the INR comparison between both groups was performed by individual proportion of time spent within the therapeutic target INR range (p=0.014); Taochek group spent the 81.6% of time into INR±0.3 target range vs 94.4% of control group (p=0.599). The number of appointments every 30 days in the Taochek group was higher than in the control group (median 1.4 vs 0.9; p<0.001). From a total of 129 determinations in the Taochek group, 6 times (5%) the dose was not proposed by the software or was overridden by the physician and in 5 time (4%) the first day schedule dose was modified. Conclusions. Our study demonstrated that OAT can be provided at least as well by computerized decision support software Taochek as by experienced physician. However the Taochek-aided dosing group required more number of appointments per patient than the control group. Taochek software is useful for the AOT control and offers an effective help to inexperienced clinical staff.

Table 1.

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<tr>
<th>The frequency of aspirin and clopidogrel resistance (platelet aggregation)</th>
<th>Hyperhomocysteinemia</th>
<th>Polymorphism</th>
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<tr>
<td>ADP 3.5µM</td>
<td>ADP 5µM</td>
<td>Collagen 2µg/mL</td>
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<tr>
<td>30-100μM</td>
<td>16-30μM</td>
<td>30-100μM</td>
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<td>5(10%)</td>
<td>9(18%)</td>
<td>3(6%)</td>
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There were no statistically significant correlations between antiplatelet drugs resistance and the plasma concentration of HCY in the C807T GPla polymorphism. Conclusions. 1. Platelet aggregation studies reveal resistance to aspirin and clopidogrel therapy in relatively few patients after myocardial infarction. 2. Platelet C807T glycoprotein Ia (GPla) polymorphism and moderate hyperhomocysteinemia are very common in survivors of myocardial infarction. 3. There is no interrelationship between resistance to aspirin and clopidogrel therapy and platelet C807T glycoprotein Ia polymorphism or homocysteine plasma concentration.
0910
NUMBER AND MIGRATORY ACTIVITY OF CIRCULATING ENDOTHELIAL PROGENITOR CELLS IN PATIENTS WITH VENOUS THROMBOEMBOLISM

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Backgrounds. Endothelial progenitor cells (EPC) derived from bone marrow are believed to support the integrity of the vascular endothelium. The number and function of EPC correlate inversely with cardiovascular risk factors, but the prognostic value associated with circulating endothelial progenitor cells has not been defined. Aims. We hypothesized that the level of EPC may contribute to the pathophysiology of venous thromboembolism (VTE). Methods. EPCs were determined in patients with VTE (n=52) and in a control group (n=47) by fluorescence-activated cell-sorting (FACS) analysis. Cells that were positive by flow cytometry for CD34/DR/AC133 within the lymphocyte population were characterized as EPCs. Results. Patients with VTE showed markedly decreased numbers of EPC (45.7%); and colony formation (74.8%) when compared with the controls (p<0.001). These findings were corroborated by 29.8% decrease in EPC migratory function in response to vascular endothelial growth factor (VEGF) (p=0.039) and 47.6% decrease in EPC incorporation into human umbilical vein endothelial cells (HUVEC) (p<0.001). CONCLUSION: The number of circulating EPC was significantly lower in patients with VTE than in normal group under the same burden of risk factors (p<0.001). The number of circulating EPC was significantly lower in patients with VTE than in normal group under the same burden of risk factors (p<0.001). Our data strongly suggest that dysfunction of circulating EPC has a role in the progression of cardiovascular complications in these patients.

0912
SOLUBLE P-SELECTIN LEVELS IN DIABETES MELLITUS PATIENTS WITH CORONARY ARTERY DISEASE

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Backgrounds. Type 2 (non-insulin-dependent) diabetes is associated with a marked increase in the risk of coronary heart disease. Platelets play a significant role in coronary artery disease. Soluble P-selectin is an index of platelets activation. Aim of the Work: is to assess the soluble P-selectin levels in Coronary artery disease. Aim. is to evaluate the levels of P-selectin in coronary artery disease in order to determine its clinical significance. Methods. Soluble P-selectin levels were measured by ELISA in the peripheral blood of 55 diabetic patients with coronary artery disease [21 acute myocardial infarction (AMI), 20 with unstable angina (UA)], 14 with stable angina (SU)], 20 patients with diabetes mellitus without coronary artery disease (DM without), and 10 healthy controls. Results. Soluble P-selectin level was significantly higher in patients with AMI (Mean±SD: 29.3±13.0 ng/mL), than those with UA (41.5±15.2 ng/mL), SU (92.1±7.67 ng/mL), DM without (89.8±7.1 ng/mL), and healthy control (69.1±4.5 ng/mL) (p<0.001). In patients with US, sP-selectin was found to be significantly elevated as compared to the SU, DM without and control group. The sP-selectin was not significantly different in DM without as compared to controls. The sP-selectin levels were correlated to the duration of diabetes mellitus(R= 0.35, p=0.03). Moreover, sP-selectin level was significantly higher in AMI patients with recurrent anginal attack as compared to that in those with single attack (P 0.041). Multivariate analysis revealed that sP-selectin levels at presentation had high adverse influence on coronary artery insult compared to LDL cholesterol level, degree of hypertension. Conclusion. Measurement of soluble p’selectin level may be helpful marker of impending coronary artery insult in diabetic patients.

0913
HR2 HAPLOTYPE IN PATIENTS WITH VENOUS THROMBOEMBOLISM IN LEBANON

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Backgrounds. Venous thromboembolism (VTE) occurs secondary to a number of hereditary and acquired disorders of hemostasis. A recently recognized polymorphism in factor V gene (His1299Arg; named HR2) has been reported to be a possible risk factor for the development of VTE. This polymorphism varies among different ethnic groups in different parts of the world. The significance of HR2 has not yet been tested in VTE patients in Lebanon. Aims. The aim of this study is to assess the possible risk of HR2 haplotype in VTE patients in Lebanon. Methods. 50 patients (27 males and 23 females) and 125 healthy subjects (72 males and 53 females), all being of Lebanese origin, were examined for HR2. The average ages for the patients and controls were 48.4±20.2 years and 35.4±18.6 years, respectively. The DNA was extracted using the FEL-FREEZ extraction kit (PEL-FREEZ, DYNAI, USA) and stored at -80°C for later use. The CVD Stripsay assay (ViennaLab, Austria) was used and its protocol was followed exactly as stated by the manufacturer. This assay screens for several gene mutations including factor V H1299R (HR2). Briefly, in vitro, the different gene sequences are simultaneously amplified and biotin-labeled in a single amplification reaction (Multiplex). The thermocycler program consists of an initial step of 94°C for 2 minutes, followed by 35 cycles of 94°C for 15 seconds, 58°C for 30 seconds, and a final extension step of 72°C for 3 minutes, followed by a final extension step of 72°C for 5 minutes. Finally, the amplification products are selectively hybridized to a test strip which contains allele-specific (Wild type and Mutant) oligonucleotide probes immobilized as an array of parallel lines. Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and color substrates. Results. Data showed that 11 patients and 13 healthy subjects had HR2 haplotype (all were heterozygous for the mutation), with a prevalence of 22.4% and 10.4%, respectively (p = 0.04). 36 patients (72%) had deep venous thrombosis (DVT), 7 patients (14%) had pulmonary embolism (PE), and 7 patients (14%) had both DVT and PE. Furthermore, among the patients who had the HR2 haplotype, 2 patients had Mutant Factor V G1691A mutation, 2 were homozygous for the MTHFR C677T mutation, and none had the prothrombin G20210A mutation. Conclusions. In Lebanon, the prevalence of HR2 is significantly high among patients with VTE with an allelic frequency of 0.11. This haplotype has a 2.4-fold greater risk of developing VTE. Moreover, it may coexist with other thrombophilia genetic mutations. Further studies are needed to be conducted in the Lebanese population in order to assess whether it is recommended to screen patients with VTE for the HR2 haplotype.

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0914  
PLATELET GLYCOPROTEIN IA C807T POLYMORPHISM AS A RISK FACTOR FOR CORONARY ARTERY DISEASE: A META-ANALYSIS

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Background-Aims. Platelet plays a crucial role in the pathogenesis of arterial occlusive disorders and platelet-dependent thromboembolism is considered an underlying mechanism in the pathogenesis of Coronary Artery Disease (CAD). The glycoprotein (GP) Iα/IIa, also known as integrin α2β1, is an important mediator of the adhesion of platelets to fibrillar collagen. Several case-control studies have investigated the importance of the α2 gene (GPIa) C807T polymorphism, a genetic marker of integrin α2β1 surface levels, as a risk factor for CAD, but the research findings were controversial. CAD continues to be the major cause of morbidity and mortality in the Western world. Hence, we carried out a meta-analysis in order to determine the importance of this polymorphism as a risk factor for CAD. Methods. Nineteen studies with data on the contribution of GPIIb/IIa polymorphism to coronary risk were identified through a comprehensive MEDLINE search up until October 2005. We used random-effects models to analyse data on studies that specifically examined cases with CAD including Myocardial Infarction (MI) and patients with only MI (including those with Acute Coronary Syndrome (ACS)). Results. The β versus the T allele contrast in the CAD group yielded an OR of 0.999 (95% CI: 0.997-1.004). The combined estimate was also insignificant when we performed the analysis in studies involving cases either with MI or with an ACS (OR: 1.013; 95% CI: 0.992-1.039). Similarly, comparing the C with the T homozygotes in the CAD group, we derived a non-significant OR (OR, 1.054; 95% CI: 0.988-1.236) and all other comparisons (CC genotype versus the others or TT genotype versus the rest) did not suggest any gene-disease association. There was no evidence of studies heterogeneity and publication bias might have not influenced the magnitude of the effect. The results remained unaffected when we fitted meta-regression models including variables such as age, risk level, gender, geographical origin, and smoking habits. Conclusions. We failed to show that the C807T polymorphism of the β2 gene could influence susceptibility to CAD either as an independent factor or in combination with any conventional risk factor.

0915  
CORRELATION OF PLATELET GLYCOPROTEIN IA C807T POLYMORPHISM AND RISK FOR CEREBROVASCULAR DISEASE: A META-ANALYSIS

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Background- Aims. Platelets are crucial in primary haemostasis and their adhesion to damaged vessel wall is mainly mediated by the collagen receptor glycoprotein (GP) Iα/IIa, known as integrin α2β1. Besides limiting blood loss at sites of tissue trauma, platelet thrombi are also responsible for the obstruction of diseased vessels, resulting in ischemia and infarction of vital organs. Although the C807T single nucleotide polymorphism of integrin α2 gene (GPIa) correlates with increased platelet surface levels of the integrin α2β1, the results concerning the association of this genetic variant with ischemic stroke have been controversial. In order to clarify this association, we performed a meta-analysis of published data regarding this issue. Methods. Seven studies providing data on the contribution of GPIIb/IIa polymorphism to the development of ischemic stroke were found through PubMed search. For the analysis of data, we used random effects models and meta-regression. Results. The pooled frequency of the T allele was 36.33% in cases and 37.01% in controls, while the T versus the C allele contrast gave an OR of 1.11 with a 95% CI 0.827-1.499. Furthermore, comparing the T homozygotes with the C homozygotes, we derived a non-significant OR (OR, 1.36; 95% CI: 0.637-2.887). Similarly, the two other contrasts (CC genotype versus the others or TT genotype versus the rest) provided absolutely no evidence of any gene-disease association. There was significant between-study heterogeneity (p<0.05). In one of the contrasts, the difference in males' percent between cases and controls was significant in the meta-regression suggesting an improper matching with regard to sex. Conclusions. This meta-analysis failed to show any significant influence of the 807T allele on the risk of stroke neither in the group of patients as a whole nor in any relevant subgroup. However, due to the significant diversity between a small number of studies in the present meta-analysis, the interpretation of the summary effect has to be done with caution.

0916  
A NOVEL HIGH SHEAR RATE ARTERIAL THROMBOSIS MODEL IN BABOONS

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Background. Animal arterial thrombosis models plays an important part in the evaluation of antithrombotic agents. Two models have been used extensively in this regard, namely those of Hanson et al and Folts et al. Unfortunately both models have limitations such as the utilisation of artificial surfaces and low shear rates, or is technically challenging. We combined elements of both models in a new high shear rate model in baboons. Aim. The development of a high shear rate arterial thrombosis model in baboons for the evaluation of anti-platelet agents. The model should allow for platelet thrombi to be formed on an injured stented artery. Methods. An arterio-venous silicon shunt was established between the femoral artery and the femoral vein. The shunt is non-thrombogenic and allows a 3-5 fold higher shear rate. A flow probe is fitted to the tubing and the femoral artery injured by applying two overlapping occlusions of the artery for one second each using a forceps. A clamp is then placed over the injured site and adjusted to produce an occlusion of 50% of the lumen diameter. Results. Effective inhibition was, however, readily reversed by infusion of 2.2 µg/kg/min epinephrin. Effective inhibition of CRFs was seen in 2/3 animals at a dose of 100 µg/kg abxicimab and in 3/3 animals at doses of 250 and 500 µg/kg. The inhibition could not be reversed by infusion of 2.2 µg/kg/min epinephrin. Conclusion. This model is suitable for the evaluation of anti-platelet agents in baboons.

0917  
B-THROMBOGLOBULIN IN CHILDREN WITH POSITIVE FAMILY HISTORY OF CORONARY ARTERY DISEASE

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Introduction: Platelets are recognized to play a key role not only in thrombus formation in acute coronary syndromes, but also, as inflammatory cells, at the onset and progression of atherosclerosis process. Aim: To investigate whether platelets are activated in children with positive family history of coronary artery disease (CAD), contributing consequently to a premature initiation of atherosclerotic process. Methods: We studied 55 healthy children (5-15 years), 30 (16 male) with family history of CAD (group A) and 25 without family history of CAD (male (group B) who were used as a control group. B-thromboglobulin (b-TG), as a marker of platelets activation, was measured from platelets taken from peripheral blood sample in all individuals included in the study. Glucose, white blood count (WBC), platelets, total cholesterol, triglycerides (TG), HDL, LDL, sedimentation rate (SDE), CRP, PAI-1 and t-PA were also measured from blood sample. Results. Children in group A had statistically significantly higher values of b-TG compared to children of Group B (72,95±15,4 vs 42,65±9,07 ng/ml, p<0,05). Among children with positive family history of CAD boys had higher values compared...
9018
SERUM LEVELS OF SOLUBLE E-SELECTIN IN VENOUS THROMBOEMBOLISM (VTE)
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Background. The inflammatory response of the vein endothelium seems to have relevance in the acute phase of the venous thromboembolism (VTE) and involves a strong expression of some cell adhesion molecules (CAMs). The serum levels of the soluble form of the E-selectin (sE-selectin) are related with the activation of endothelial cells but the evolution of their values in a later (chronic) phase of the VTE is not known. We aim to identify any association between the sE-selectin and the VTE six months after the acute phase. Populatio n and Methods. We measured the serum sE-selectin concentrations from 194 subjects. 19 patients objectively diagnosed of venous thromboembolism (VTE) six months after the acute phase and 197 controls of similar gender and age [61.5 (12.9) years, 49.0% males] by an enzyme-linked immunosorbent assay (ELISA) method. The non-Gaussian distribution of the sE-selectin values requires the use of non-parametric statistical tests. Results. In the overall series, the level of the sE-selectin directly correlates with the waist / hip ratio (r=0.21, p<0.0001) being higher among the males [66.0 (54.5) vs. 55.5 (38.1) ng/mL, p=0.001] although showing a weak inverse correlation with the age (r=-0.135, p<0.01). The sE-selectin was independent of the body mass index (NS p). A trend to lower sE-selectin values appears among the patients [56.5 (42.6) ng/mL] versus the controls [65.0 (47.6) ng/mL] (p=0.055, Mann Whitney test). However the extreme values did not show association with the VTE (90th percentile 124.5 ng/mL; OR=1.06, NS p and 10th percentile 83.1 ng/mL; OR=1.17, NS p). The soluble E-selectin was also similar in recurrent (n=50) and non-recurrent cases (55.3 (33.4) vs. 57.0 (45.2) ng/mL) (NS p). Conclusion. The soluble E-selectin values were not clearly related with the VTE in a late phase (six months after the acute episode).

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9019
NO EFFECT OF B-VITAMIN SUPPLEMENTATION ON MARKERS OF THROMBIN GENERATION IN PATIENTS WITH VENOUS THROMBOEMBOLISM
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Backgrounds. Mild hyperhomocysteinemia is associated with an increased risk of venous thromboembolism (VTE) and other cardiovascular diseases. In vitro studies showed that homocysteine may stimulate procoagulant factors and increase thrombin generation. Folic acid and B-vitamins supplementation decrease homocysteine levels, but it is not clear whether this may interfere with its procoagulant effects. Aims. To evaluate the effect of vitamin supplementation on the homocysteine level and on markers of thrombin generation in patients with VTE. Methods. This study was a multicentre, randomized, double-blind, placebo-controlled clinical trial. We randomized 105 patients with a first event of objectively confirmed VTE, aged between 18 and 70 years, to receive either vitamin supplementation (folic acid 5 mg, vitamin B6 50 mg and vitamin B12 0.4 mg) or placebo. Blood was collected at randomization and 8 weeks after the intervention period. Results. Ninety-nine (94.3%) completed the 8-week period of treatment. There was no difference between patients with homocysteine above the highest tertile (12.6 micromol/L) and those below the lowest tertile (9.9 micromol/L) in the levels of prothrombin fragment 1+2 (median 0.73 and 0.71 nmol/L, respectively), thrombin-antithrombin complex (median 4.5 and 4.1 microg/L) and D-dimer (median 277 and 256 ng/mL). In patients treated with vitamins, there was a 29% decrease in the homocysteine levels. However, prothrombin fragment 1+2, TAT and DD levels did not change, both in the vitamin and in the placebo groups. Besides, treatment with vitamins had no effect on these markers, even in patients above the highest tertile of homocysteine. Conclusions. In patients with VTE, higher homocysteine levels are not associated with higher levels of thrombin generation markers and homocysteine reduction by B-vitamin supplementation had no effect on these markers.

9020
IMPACT OF A DIAGNOSTIC PROTOCOL FOR DEEP VEIN THROMBOSIS ON REQUESTS FOR D-DIMER ASSAYS
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Backgrounds. Sensitive D-dimer assays have been shown to be useful for decreasing the need for formal radiological imaging in the diagnosis of Deep Vein Thrombosis (DVT) in selected patients who have low pretest probability (PTP) scores. Aims. Unfortunately in our institution the PTP score was not always assessed and the D-dimer test was therefore being used to rule out DVT even in those patients who would have had a high PTP. This is inappropriate and has resulted in missed diagnoses. Methods. In Jan ’05 we introduced a diagnostic protocol in A+E, which relied on a Wells’ PTP Score (Figure 1). Patients with low PTP (0 or 1) and D-dimer value < 250 ng/L did not require radiological imaging and were discharged. The haematology laboratory was empowered to reject samples for D-dimer testing if the request form did not contain a Wells’ PTP. In contrast, patients with a high PTP (2 or 3) proceed to radiological imaging without a D-dimer test. An audit of compliance with the new pathway was assessed, and an action plan formulated to promote awareness and compliance. Results. The results were compared one year later: the number of D-dimer requests has decreased by 50% with the new protocol. There are now no apparent missed DVTs (as indicated by patients who returned to the A+E department and later were confirmed to have DVTs in the year prior to introduction of the protocol). Summary: D-dimer assays must be used in conjunction with a clinical pre-test probability score: a low PTP and a negative D-dimer can reliably exclude DVT. Prior to the introduction of this protocol we received 350 D-dimer requests per month. The introduction of a Wells’ PTP and selective use of D-dimer testing resulted in cost savings, BMS time efficiency and significant relief in manpower pressures in providing the ‘4-hour D-dimer service.’

References
1. BCSH. BJHaem, 124,15-25
0921  RELATIONSHIP BETWEEN HOMOCYSTEINE LEVELS AND MTHFR GENOTYPE, AND THEIR EFFECT ON DEEP VENOUS THROMBOSIS

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Backgrounds. High serum total homocysteine concentrations (tHcy) are suggested to be a risk factor for arterial and deep venous thrombosis. 5,10-methylenetetrahydrofolate reductase (MTHFR) is a crucial enzyme in homocysteine metabolism. The mutation MTHFR C677T renders the enzyme thermolabile and leads to elevated tHcy levels. Aim: In this study the relationship between tHcy levels and MTHFR C677T polymorphism in patients with first episode of deep venous thrombosis (DVT), as well as the assessment of hyperhomocysteinemia as a risk factor for DVT were investigated. Methods: 46 (20 men, 26 women) healthy individuals (group A) aged 41.59±14.11 years and 74 (57 men, 37 women) patients with first episode DVT (group B) aged 45.99±14.98 years were enrolled. Total homocysteine levels were determined with ACS: 180® SE Automated Chemiluminescence Systems (Bayer). MTHFR genotypes were analyzed using PCR amplification and digestion with restriction endonuclease Hinf I. The data were expressed as the mean±SD and analyzed with Student test, Univariate Analysis of Variance, Tukey test, Logistic Regression Analysis. Values of p<0.05 were considered to be statistically significant. Hyperhomocysteinemia was set at 90th percentile of tHcy levels of group A. Results. Statistically significant difference in tHcy levels between groups A and B was observed (A vs B, 12.44±4.43 vs 14.54±5.57 µmol/l, p=0.029). The frequency of alleles was 0.59±0.069 for C allele and 0.402±0.311 for T allele in groups A and B, respectively. Among the subjects with T/T genotype, higher tHcy concentrations were detected in group B than group A (T/T, A vs B, 13.07±4.67 vs 22.47±7.22, p=0.012). No important difference was found in the tHcy levels between the two groups with respect to C/C (p=0.141) and C/T (p=0.392) genotype (A vs B, C/C: 11.28±4.17 vs 13.07±3.96, C/T: 13.14±4.55 vs 14.60±5.16). There was no effect of MTHFR C677T mutant genotype on tHcy levels in group A. Total Hcy concentrations in patients (group B) with T/T genotype are statistically higher when compared to C/C (C/C vs T/T, 13.07±3.96 vs 22.47±7.22, p=0.001) and to C/T (C/T vs T/T, 14.60±5.16 vs 22.47±7.22, p=0.001) genotypes. Hyperhomocysteinemia (tHcy > 19.30 µmol/l) was observed at 8.7% (4/46) of group A and 21.6% (16/74) of group B. Logistic regression analysis indicated that only hyperhomocysteinemia is an independent risk factor for DVT (Odds Ratio=3.95, CI 95%: 1.1-14.4, p=0.037), while the genotype (p=0.268) and the interaction between genotype and hyperhomocysteinemia (p=0.568) are not risk factors. Conclusions. Our results indicated that patients with T/T genotype have higher tHcy levels when compared to healthy individuals as well as to patients with C/C and C/T genotypes. Hyperhomocysteinemia is an independent risk factor for deep venous thrombosis. The T/T genotype and the combination of hyperhomocysteinemia and T/T genotype are not related to deep venous thrombosis.

0922  ADAMTS-13 GENE MUTATION IN A PATIENT WITH THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP)

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Background. The vWF-clearing protease (ADAMTS-13) is important for maintaining the normal size distribution of vWF multimers. A severe deficiency of ADAMTS-13 activity (i.e. <5% of that of normal plasma), caused by either mutation of the ADAMTS-13 gene or by inhibitory auto-antibodies to ADAMTS-13, is associated to TTP. Aim and Methods. We describe the case of a 19-year-long chronic relapsing TTP. A 45-year-old woman was first diagnosed with TTP during pregnancy, in 1987 when she was 27 years. Subsequently, she relapsed three times in 1995, once in 1996, and once in 2000. On each episode she was treated with plasma exchange and steroid therapy, obtaining the complete remission. Results. In 2001, the ADAMTS-13 testing became available in our laboratory and we prospectively followed the patient for ADAMTS-13 activity and inhibitors. In addition, since 2005, we also tested the plasma levels of anti-ADAMTS-13 antibody. In 2004 she relapsed after starting interferon for HCV-related chronic hepatitis. Plasma exchange and steroid therapy were resumed, without achieving a durable remission, as she relapsed after one month. She was then given chemotherapy (i.e. fludarabine, cyclofosfamide) and rituximab, without significant response. From January 2005 on, she is receiving periodic plasma infusions on the basis of platelet count and is continuing on this regimen so far. The measurement of ADAMTS-13 activity from 2001 showed a severe deficiency (<5%) of this protease during clinical remissions and upon relapses. No significant plasma inhibitory activity was found by mixing studies. The retrospective quantification of auto-antibodies by ELISA revealed no significant levels of anti-ADAMTS-13 antibodies. The patient was then identified as a possible carrier of a true constitutive ADAMTS-13 deficiency. The DNA analysis of this patient detected homozygosity for the 5428 C>T in exon 25 of the ADAMTS-13 gene, which predicts the R1123C exchange in the TSP1-8 domain. The inherited nature of severe ADAMTS-13 deficiency was established by family analysis. Conclusions. This mutation has been previously linked to Upshaw-Schulman syndrome (USS), a congenital chronic relapsing form of TTP, characterized by neonatal onset, response to fresh plasma infusions, and frequent relapses. Differently, in our patient the onset of clinically overt disease manifested in the adult age during pregnancy, thus supporting the hypothesis that additional precipitating factors may determine the phenotypic manifestation of this mutation.

0923  IS THE VARIABLE CLINICAL PRESENTATION IN HEREDITARY TTP THE RESULT OF DIFFERENCES IN RESIDUAL ADAMTS13 ACTIVITY?

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Backgrounds. Hereditary TTP (Upshaw Shulman syndrome, USS) is due to homozygous or compound heterozygous ADAMTS13 gene mutations. Analysis of patient histories revealed a striking age-dependent clustering of the first TTP attack. While half of the patients develop clinical signs of acute TTP immediately after birth or in early childhood (early onset), the other half remains asymptomatic into early adulthood and suffers from a first acute TTP episode at the age of 20-40 years (late onset) and this clinical pattern is often very similar in affected siblings. So far, no correlation between the clinical phenotype, i.e. disease severity, organ tropism and the underlying genotype is discernable. In analogy to the situation in hemophiliacs (distinction between <1% and 1-5% determination of residual ADAMTS13 activity could help to elucidate the variable clinical presentation. As VWF levels increase with age to the situation in hemophiliacs (distinction between <1% and 1-5% determination of residual ADAMTS13 activity could help to elucidate the variable clinical presentation. As VWF levels increase with age to
after the last plasma infusion were analyzed. ADAMTS13 activity was determined by a new fluorescence resonance energy transfer assay using a synthetic, truncated 73-amino-acid VWF peptide as a substrate (FRET-VWF73 assay; Kokame et al. Br J Haematol. 2005;129:95-100), which was modified in order to reliably distinguish between 0% and 1% of ADAMTS13 activity, which had not been possible with the older assays.

Results. Half of the USS patients (14/28) had an ADAMTS13 activity <1%, by FRET-VWF73; 11 patients displayed a residual activity between 1-5% and in three instances ADAMTS13 activities lay between 5 - 8%. Attempts to link patients histories with their ADAMTS13 activities failed, as patients from both clinical groups (early vs. late onset) had ADAMTS13 activity values <1% or in the range of 1-5%. Conclusions. Differences in residual ADAMTS13 activity are apparently not accountable for the documented age-related presentation in USS. It is thus likely, that other disease-modifying genetic (i.e. blood group, VWF levels) or environmental factors affect the phenotype.

0924
INCIDENCE AND LABORATORY FEATURES OF THROMBOCYTOPENIA IN 43 PATIENTS WITH VON WILLEBRAND DISEASE TYPE 2B: CORRELATION WITH MOLECULAR DEFECTS AND ACQUIRED MODIFICATIONS OF VWF

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Backgrounds. Von Willebrand type 2B (VWD) is an inherited bleeding disorder caused by abnormal von Willebrand factor (VWF) that displays increased affinity to the platelet glicoprotein 1b α (GpIba). VWD 2B is due to a group of mutations clustered within VWF A1 domain and is characterized by binding of its high molecular weight multimers (HMW) to platelets often resulting in moderate-mild thrombocytopenia. Even though there are many case reports on thrombocytopenia associated with VWD 2B, retrospective and prospective studies in a large cohort of patients are not available. Aims and design of the study. To determine incidence and laboratory features of thrombocytopenia in VWD 2B, we have prospectively observed our cohort of 43 patients (18 families) previously characterized by VWF mutations. Methods. Data of platelet count with mean platelet volume (MPV) and morphologic evaluation of the blood smear to search for giant platelets or aggregates were associated with the history of physiologic or pathologic stress conditions such as pregnancy, infections, surgery or use of DDAVP. All patients were characterized by ristocetin induced platelet agglutination (RIPA) in the Platelet Rich Plasma (PRP), ristocetin cofactor activity (VWF:Kc) with VWF antigen (VWF-Ag), multimeric structure of VWF. Mutations within VWF A1 domain were searched for and confirmed by sequencing exon 28.

Table 1.

<table>
<thead>
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<th>Mutation</th>
<th>RIPA</th>
<th>VWF-Ag</th>
<th>Low plt. (&lt;140 x 10^3)</th>
<th>Plt morphology</th>
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<td>4</td>
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<tr>
<td>V13410 (4)</td>
<td>0.67</td>
<td>43</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>R1341L (1)</td>
<td>0.70</td>
<td>43</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Results. Among 43 VWD cases, a platelet count< 140,000 was found at baseline in only 11 (26%), but was observed after stress conditions in 34 cases (79%); no reduced platelet counts was found in 9 patients (21%) from two different families (R1308L, R13410). An increased MPV was found in 13 cases but giant platelet and aggregates in only 6 cases. All the phenotypic features were correlated to VWF mutations. Conclusions. Based on these results, thrombocytopenia can be associated in most VWD 2B patients, especially when high levels of mutant VWF are triggered by physiologic and pathologic stress conditions. However, not all VWD 2B show thrombocytopenia and a relatively high degree of heterogeneity of this phenomenon occurs within patients characterized by the same molecular defects.

0925
DIFFERENT ACTIVATION STATUS IN PERIPHERAL VERSUS SPLENIC T LYMPHOCYTES IN IMMUNE THROMBOCYTOPENIC PURPURA PATIENTS

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Backgrounds. Adult idiopathic thrombocytopenic purpura (ITP) is an autoimmune disorder characterized by enhanced splenic destruction of the platelets, through autoantibodies binding to membrane glycoproteins. B lymphocytes secreting antiplatelet antibodies are considered as the major mechanism in ITP. Nevertheless, cellular immunity could have a role in ITP pathophysiology. Recently, autoreactive CD4+ T cells directed against membrane platelets proteins have been identified and another group pinpointed the implication of CD8+ cytotoxic T lymphocytes for a direct cell-mediated lysis of autologous platelets in active ITP. Aim: We explored activation status of both CD8+ and CD4+ T-lymphocyte subsets in chronic ITP patients in peripheral blood and spleen when splenectomy was indicated. We surmised that different T-lymphocyte activation status could reflect different pathogenic mechanisms involved in platelet destruction. Methods. Fifty four patients with chronic ITP were enrolled prospectively in the present study and compared to 46 normal healthy volunteers. Among ITP group 17 patients had a splenectomy. Phenotypic analysis was conducted on peripheral T-lymphocytes and compared to T-lymphocytes obtained from post traumatic splenectomy. Flow cytometry was used to evaluate T-lymphocyte HLA-DR membrane expression. We used Wilcoxon-Mann-Whitney test to compare continuous variables, as they did not present normal distributions. We performed a Bonferroni adjustment to prevent the raise type I errors due to multiple testing between groups. Results. All 46 patients fulfilled ASH criteria for chronic ITP. Their ages ranged from 16 to 79 years with a median age of 49 years. Sixteen patients were male and 38 female. Median platelet count was 42500/mm3 (1000-186000/mm3). The percentage of CD8+ DR+ peripheral T-lymphocytes was significantly higher in ITP patients (10.79% vs 7.20%, p=0.004), with predominance for activated CD4+ subsets (6.12 vs 2.71, p=0.001) compared to activated CD8+ T cells (7.6 vs 3.86, p=0.0045). This activation was correlated with platelet counts for both subsets (r=0.16) (Figure 1).

Nevertheless, this activation status was not correlated to treatment efficacy nor prognosis. Study of splenic T-cell subset activation reveals different results. Indeed, only CD3+CD8– splenic lymphocytes were found activated in ITP spleen, compared to controls (14.5% [4.96 - 32.65] vs 7.13% [2.55 - 13.27], p=0.008). Conclusion: From the present study we can conclude that there is a correlation between the severity of ITP and the increased percentage of activated T lymphocytes. Nevertheless, there is no correlation between this activation and prognosis. Interestingly, refractory ITP are characterized by an increase of splenic activated CD8+ that could be involved in platelet destruction. This observation may corroborate the possible implication of different pathogenic pathways involved in ITP.
Prevalence and relevance of heparin-induced antibodies in LMWH-treated pregnant women

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Backgrounds. Heparin-induced antibodies (HI-Ab) and heparin-induced thrombocytopenia (HIT) have been demonstrated less frequent due to LMWH than UFH, however, this has been questioned in non-surgical patients more recently. So far no HIT cases in LMWH-treated pregnant women have been reported, whereas thrombocytopenia of other aetiology may develop during pregnancy. Aims. The purpose of this study was to investigate whether women treated with LMWH during pregnancy present with or develop HI-Ab and whether HI-Ab are of clinical relevance. Patients and Methods. 111 women with a history of thrombosis (n=71), risk factors for thrombosis (n=40) and/or recurrent fetal loss (n=19) completed 121 pregnancies and were treated with LMWH or danaparoid (nadoparin n=101, dalteparin n=9, enoxaparin n=9, certoparin n=6, danaparoid n=5) during pregnancy and for 4 to 6 weeks after delivery. Inherited thrombophilia was diagnosed in 68/111 (61.3%) patients, 40 of them (59%) had experienced thrombosis. LMWH was initiated between week one and 30 of pregnancy depending on history and thrombosis risk. Development of HI-Ab was investigated by heparin-platelet-factor-4-ELISA (Asserachrom HPIA, Roche, Germany) in 4-8 week intervals. Positive ELISA results were additionally tested by the heparin-induced platelet-activation-assay (HIPAA). Platelet count was monitored once a week during the first 6 weeks of LMWH treatment and thereafter every 4-8 weeks. All measurements of HI-Ab were performed after termination of heparin treatment unless platelet count dropped for more than 50% or thrombocytopenia (< 150 G/l) developed. Results. HI-Ab were detected in 6/121 (5.0%) pregnancies by ELISA, none was positive in the HIPAA. Four of the six patients had a history of previous UFH exposure. Four patients had low (OD <1.0) and two intermediate HI-Ab-titres (OD 1.0-2.0). Cut-off OD 0.43-0.67 depending on the batch). In two patients with low titres (OD 0.63 and 0.56) HI-Ab were already present before LMWH treatment and normalised in one during LMWH administration. Interestingly, two patients with repeated pregnancies revealed HI-Ab during the first but not the second pregnancy. None of the six patients with HI-Ab developed thrombocytopenia or a platelet drop >50%. However, in 8/115 (7.0%) pregnancies without HI-Ab mild thrombocytopenia (range 105-147 G/l) became apparent mostly in the third trimester. Local allergic reactions occurred in 7.2% of patients and required change of anticoagulation. Conclusions. Heparin-induced antibodies in pregnancies treated with LMWH are detectable with low frequency and usually low titres. Antibodies might in part be due to previous UFH exposure. None of the HI-Ab-positive patients developed thrombocytopenia, hence, the risk of LMWH-induced thrombocytopenia in pregnant women appears to be very low. Most thrombocytopenias are pregnancy-associated and of mild type, however, might be difficult to distinguish between heparin and other factors as cause.

Increased platelet-monocyte and platelet-neutrophil complex formation in primary Raynaud phenomenon and in Raynaud phenomenon secondary to systemic sclerosis

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Backgrounds. Although it was suggested that platelet activation in systemic sclerosis (SSc) was secondary to microvascular damage, there is also data that it is the primary event. As there is both platelet and leucocyte activation in both primary raynaud phenomenon (RP) and RP secondary to SSc, it is possible that increased platelet-leucocyte interaction contributes to coagulation system alterations in RP patients. It was stated that platelet-leucocyte interaction was an important factor in the pathogenesis of vascular ischemic syndromes. In addition, active platelets secrete microparticles (PMP) with procoagulant activity. This is the first study to evaluate platelet-monocyte complexes (PMC, platelet-monocyte-activatingplexes (PMP) and PNC and PMP in RP, Aims. We evaluated platelet activation markers and PMP, PNC in patients with primary RP and in RP secondary to SSc. Methods. We utilized whole blood flow cytometry to quantify the expression of CD62P, PMP, and the percent-ages of PNC and PNC in primary RP patients and in SSc patients with secondary RP. Results. We included 16 consecutive SSc patients with sec-ondary RP (15F, 1M, mean age:44.3), 12 primary RP patients (10F, 2M, mean age:33.6), and 18 healthy subjects (16F, 2M, mean age: 39.2) as our control group. The mean duration of symptoms in primary RP patients was 7.2 years, and it was 8.4 years in patients with secondary RP. CD62P expression in SSc patients with secondary RP was significantly higher than in primary RP patients and in controls (p values, respectively, 0.017 and 0.004). PMC and PNC were significantly higher in both primary and secondary RP groups than in controls (all p values <0.001). Although PMP level in primary RP group was higher than in controls, this difference was not significant (p=0.1). Table 1. PMP level in SSc patients with pulmonary artery hypertension (PAH) was significantly higher when compared to those without PAH (4±0.5 vs. 3.4±0.6, p=0.049). The other parameters evaluated in SSc patients did not significantly differ between groups with or without digital ulcers or loss, those with or without interstitial lung disease, aspirin-users and nonusers (p>0.05). In addition, PMP level had a correlation with pulmonary artery pressure (r=0.59, p=0.017). There was a trend towards higher PMP levels in the anti-centromere-positive group (4±1.4 vs. 3.5±0.8, p=0.1). In primary RP patients, PMC level had positive correlations with PNC (r=0.68, p=0.015) and CD62P (r=0.61, p=0.035). In SSc patients with secondary RP, PMC level had a positive correlation with PNC (r=0.88, p<0.001); CD62P level had a negative correlation with PMP (r=-0.5, p=0.046). In 4 patients administered iloprost, the mean CD62P level decreased significantly (4±1.7 vs. 3.4±0.5, p<0.05); PMC (62±20 vs. 50±10) and PNC levels (34±11 vs. 27.5±6.5) regressed nonsignificantly (p values >0.05). Conclusion. Our results suggest that platelet-leucocyte complex formation is increased in RP. In addition, it provides evidence that there is ongoing platelet activation and platelet-leucocyte interaction even in patients despite anticoagulant therapy. We suggest it is important to consider as it might have potential therapeutic implications with respect to the use of antplatelet drugs in these patients.

Table 1. CD62P, PMC, PNC, PMP in RP patients.

<table>
<thead>
<tr>
<th></th>
<th>Primary RP</th>
<th>RP secondary to SSc</th>
<th>Controls</th>
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</thead>
<tbody>
<tr>
<td>CD62P (%)</td>
<td>6.7±3.8</td>
<td>17.7±13.9</td>
<td>6.1±8.6</td>
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<tr>
<td>PMC (%)</td>
<td>67±20.1</td>
<td>64.1±27.3</td>
<td>22.4±9.1</td>
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<tr>
<td>PNC (%)</td>
<td>37.2±22.8</td>
<td>33.3±16.6</td>
<td>9.2±4.1</td>
</tr>
<tr>
<td>PMP (%)</td>
<td>4.2±1.4</td>
<td>3.7±0.7</td>
<td>3.6±0.5</td>
</tr>
</tbody>
</table>

CD62P: SSC is different from in primary RP and controls (p values 0.017 and 0.004); PMC: primary and SSC are different from controls (all p values <0.001); PNC: primary RP and SSC are different from controls (p values 0.001 and < 0.001).

Low rate of long lasting remissions after successful treatment of immune thrombocytopenia with rituximab

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Backgrounds. Immune thrombocytopenic purpura is characterised by peripheral platelet destruction due to autoantibodies derived against surface glycoproteins. Management of patients with autoimmune thrombocytopenias is difficult, relapses are common. Recent studies have shown that the anti-CD20 antibody rituximab is effective in the treatment of relapsed and refractory patients. Aims. The aim of this study was to evaluate rituximab therapy in ITP patients in our institution. Methods. We report the results of a retrospective analysis of rituximab treatment in 14 patients with immune thrombocytopenic purpura. All patients had received 1-7 lines of previous therapies, 4 had undergone previous splenectomy. Rituximab was administered at the standard dose of 375 mg/m² once per week with a total of 4 infusions (range 1-4). Results. The overall response rate was 64%, 7 of 14 patients (50%) achieved a complete remission (platelet levels >100x10/L). 2 of 14 patients (14%) showed a partial remission (platelets >50x10/L). 5 patients did not respond to the therapy. The median time to response after the start of the rituximab treatment was 4 weeks (range 1-4). Three patients (21%) had a long lasting remission and ongoing remission up to 156 weeks. Responding patients remained in remission for a median period of 8 weeks (range 10 days - 36 months). All of the 4 splenectomized patients had a complete remission after rituximab therapy, with 2 long lasting remissions for 26 and 156 weeks. Summary/Conclusions. Our observations show that rituximab treatment represents a well tolerated and effective therapy for patients with autoimmune thrombocytopenias even in previously refrac-
**0929**

**AUTOIMMUNE THROMBOCYTOPENIA: FLOW CYTOMETRIC DETERMINATION OF PLATELET-ASSOCIATED CD154/CD40L AND CD40 ON PERIPHERAL BLOOD T AND B LYMPHOCYTES**

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**Background and Objectives.** The CD40-CO40L system has pleiotropic effects in a variety of cells and biological processes including immune response. Within the immune system, these molecules represent a critical link between its humoral and cellular arms. Immune or idiopathic thrombocytopenic purpura (ITP) is an autoimmune disorder characterized by antibody-induced platelet destruction and clearance because of anti-platelet autoantibodies, which bind to circulating platelets resulting in their destruction by the reticuloendothelial system. Despite its clinical importance, the diagnosis of ITP is one of exclusion, thus, inevitably associated with potential difficulties. CD40 is a cell surface receptor that belongs to the tumor necrosis factor receptor (TNF-R) family, and was first identified and functionally characterized on B lymphocytes. CD40-ligand (CD40L/CD154), a member of the TNF superfamily, is a cell membrane molecule expressed on activated CD4+ T lymphocytes and is essential for the T cell-dependent activation of B lymphocytes. Therefore it is now thought that CD40-CD40L interactions play a more important role in ITP immune regulation. Design and Methods. The expressions of CD4 and CD40 on peripheral blood (PB) T and B lymphocytes, respectively, were measured using the technique of flow cytometry. An antigen-specific assay for platelet-associated antibody CD154 (CD40L) on CD4 T lymphocytes and for CD40 on CD19- B lymphocytes was tested in 30 children patients with acute ITP, 30 adult patients with chronic ITP, and in 20 age- and sex-matched healthy controls. Results. The expressions of CD4+CD154+ and of CD4+CD154+/CD4- on PB T lymphocytes, and of CD19+CD40+ and of CD19+/CD40+ on PB B lymphocytes were significantly higher in acute and chronic ITP patients compared to controls, and in acute patients compared to chronic (<0.001). Conclusions. CD40-40L interaction plays an important role in the pathology of certain autoimmune diseases. ITP is an autoimmune disease characterized by increased platelet destruction caused by anti-platelet autoantibodies, which mainly target a platelet surface antigen. It is speculated that platelet-associated CD154 is competent to induce the CD40-dependent proliferation of B lymphocytes. Therefore, platelet-associated CD154 expression is increased in ITP patients and is able to drive the activation of autoreactive B lymphocytes in this disease. These findings are particularly useful for clarifying the pathogenic process in ITP patients and for developing a therapeutic approach that blocks pathogenic anti-platelet antibody production. Blockade of the CD40/CD154 signal is a potential immunomodulatory strategy for T-cell-mediated diseases, and many findings suggest that CD40/CD154 blockade therapy is potentially effective for ITP through selective suppression of autoreactive T and B lymphocytes to platelet antigens.

**0931**

**EFFICACY AND SAFETY OF IVIG31 GRIFFOLS (HUMAN INTRAVENOUS IMMUNOGLOBLIN) IN PATIENTS DIAGNOSED WITH IMMUNE THROMBOCYTOPENIC PURPURA**

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Intravenous immunoglobulin (IVIG) therapy is a useful therapeutic approach in patients with chronic idiopathic/immune thrombocytopenic purpura (ITP) in whom the platelet count has to be rapidly increased to prevent bleeding or prior to surgery. IVIG31 Griffols is a highly purified, unmodified human IgG product whose manufacturing process follows the same basic principles of FebycoTM (another IVIG manufactured by Griffols in clinical use since 1992). The main differences between both processes are how purification steps are sequentially arranged, and the introduction of two specific steps to inactivate/remove any potential contaminating pathogen (solvent-detergent treatment and sequential nanofiltration through 35 and 20 nm pore size filters), as additional viral elimination steps to pasteurization, already present in FebycoTM. Essentially IVIG31 Griffols is prepared from fraction II-III of Cohn’s fractionation and the purification of IgG is performed by means of sequential polyethylene glycol precipitations. Further reduction down for all remaining potential impurities is achieved through ion exchange chromatography with DEAE resins. Finally, it is formulated with sorbitol (5%) as stabilizer. An open, prospective, multicentre study was planned to investigate the efficacy and safety of IVIG31 Griffols in 20 adult patients with chronic ITP (at least 6 months after diagnosis). It was designed in accordance with the European Union guidelines from the EMEA for such trials. A total of 19 adult patients with chronic ITP in acute phase (platelet counts below 20 × 10^9/L) were treated with the study drug. Patients received a total dose of 0.4 g/kg body weight for 5 consecutive days. Primary efficacy endpoint was the proportion of patients who reached a platelet count equal or > 50 × 10^9/L. The time taken for the platelet count to reach the target level since first dose of IVIG31 Griffols and duration of response

**Table 1.**

<table>
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<tr>
<th>Median</th>
<th>Range</th>
<th>Std. Dev.</th>
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<tbody>
<tr>
<td>PAC-1</td>
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<td>0.3-0.7</td>
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<tr>
<td>PAC-1 ADP</td>
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<td>0.7-5.7</td>
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<tr>
<td>ΔMFI</td>
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<tr>
<td>ΔMFI ADP</td>
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</tr>
<tr>
<td>CD62 (%)</td>
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<td>4.4-18.0</td>
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<tr>
<td>CD62 ADP</td>
<td>26</td>
<td>11.3-57.4</td>
</tr>
<tr>
<td>ΔCD62 p (%)</td>
<td>14</td>
<td>1.7-44.1</td>
</tr>
</tbody>
</table>

**0930**

**AGONIST INDUCED PLATELET ACTIVATION IN A HEALTHY POPULATION. STUDY BY FLOW CYTOMETRY**


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**Aims.** To design a protocol to establish normal parameters of platelet (Pit) activation in our population using whole blood flow cytometry assays, and study the variability of responsiveness to an agonist among healthy volunteers. **Material and Methods.** 25 healthy blood donors were included in the study, none of them showed diabetes, hypertension or pharmacological treatment. Blood samples were drawn using standard phlebotomy techniques to obtain a 4.5 mL vial containing 3.8% citrate with a 16G needle. Samples were collected in conditions to avoid stasis and prevent non-physiological Pit activation. To avoid possible observer bias, blood samples were coded and blinded. The blood was carefully mixed and diluted in Tyrode’s buffer (not diluted in monocytes (mon) and neutrophils (nt)-binding platelets study), and then separated into six polypropylene tubes (3 baseline and 3 adding ADP). To detect Pit response to an agonist (activation) we added a 100 μM ADP concentration. The surface expression of Pit receptors was determined by flow cytometry using the following monoclonal antibodies: anti-CD41 (Gp IIb/IIIa) FITC and PE, anti-CD62P (P-selectin) PE, anti-CD11b FITC, anti-CD14 PCS (Immunotech, Marseille, France) and PAC-1 (activated Gp IIb/IIIa) FITC (BD Biosciences, San Jose, CA, USA). The samples were analyzed on a Coulter® EPICS XL-MCLTM. All parameters were collected in list mode fields and then analyzed. PAC-1 was expressed in mean fluorescence units (MFI) of total plt population. CD62p and leukocytes positive for pTs were expressed in percentage (%). Descriptive statistics and correlation test were also studied. Results. Blood samples from 3 females and 22 males with a median age of 40 years (range: 25-61) were studied. See descriptive statistics in table below. We found a significant correlation coefficient (r=0.837) between P-selectin expression and PAC-1 binding. We didn’t find correlation between age and activation parameters. Conclusions. 1) Our data indicate that ADP induced plt activation varies considerably from one individual to one, as observed by other groups. 2) In healthy adults we demonstrate that the expression of P-selectin (% granules release) is strongly correlated to the binding of PAC-1 (conformational change) according to the results of other series.
were also determined within 1 month after first infusion. Regression of haemorrhages was documented during the first 14 days of follow-up. Safety parameters including adverse events (AEs), laboratory determinations and vital signs were regularly monitored. The follow-up of patients ended 3 months after first dose of IGIV3I Grifols to determine any change in viral markers for HIV, HCV, HBV and HAV. An interim analysis from available results of 8 out of 20 patients is presented. A patient was withdrawn from the study after confirmation of secondary thrombocytopenia. A total of 5 patients (71%) responded to the study drug. The mean time to platelet response was equal or <34 days (SD=1.1) and the mean duration of response was equal or >10.0 days (SD=7.9). Haemorrhagic symptoms compared with baseline improved in all seven patients (6/7). Five out of 8 treated patients presented a total of 12 AEs potentially related to the study drug (8 mild and 4 moderate). Headache and fever (4 cases each), changes in blood pressure (3 cases) and decrease in heart rate (1 case) were AEs potentially related to study drug. The results show that IGIV3I Grifols is effective, well tolerated and safe in the treatment of adult patients with chronic ITP.

**0932**

**RITUXIMAB IN REFRACTORY IDIOPATHIC THROMBOCYTOPENIC PURPURA**

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**Background.** Rituximab, a chimeric anti-CD20 monoclonal antibody effective in B-cell depletion, may be useful in autoimmune disorders interfering with the production of auto-antibodies. Aims. To investigate the efficacy of Rituximab in patients with resistant ITP. Patients and Methods. Eleven adult ITP patients (2 males, 9 females; median age 46.3 years, 28.6-67.8) were treated with Rituximab (375 mg/m<sup>2</sup>/weekly for four doses). Median time between diagnosis and start of Rituximab was 4.1 years (0.2-35.1 months). All patients had already received at least two lines of therapy (median 3; 2-6); prednisone, pulsed high-dose dexamethasone, azathioprine, immunoglobulins, interferon or splenectomy. At the start of Rituximab, the median platelet count was 10×10<sup>9</sup>/L (5-20×10<sup>9</sup>/L). Response definitions: complete response (CR), platelet count ≥150×10<sup>9</sup>/L; partial response (PR), >50 <150×10<sup>9</sup>/L; minimal response (MR), >20 50×10<sup>9</sup>/L; no response (NR) ≤20×10<sup>9</sup>/L. After completing therapy, patients were evaluated for platelet count after 1 and 3 months, and thereafter every 3 months until relapse or start of a different treatment. Peripheral blood lymphocytes were evaluated by flow-cytometry as CD19<sup>+</sup> cells before treatment, 1 and 3 months after stopping therapy, and then every 3 months up to recovery. Results. One month after Rituximab therapy, 5 responses (1 CR, 3 PR, 1 MR; 45%) and 6 NR (55%) were observed. Two relapses occurred 5 and 18 months after response. The median follow-up of all treated patients is 8.7 months (1.8-31.1), while the median follow-up of all responsive patients is 13.7 months (2.6-18.7). Before starting therapy, 9/11 patients were evaluable for flow-cytometry studies. The median baseline value of peripheral blood CD19<sup>+</sup> B-cells was 128×10<sup>3</sup>/L (58-571). One month after completing therapy, 6/8 evaluable cases showed absence of CD19<sup>+</sup> cells and 2/8 showed a count of 9 and 4.4×10<sup>3</sup>/L CD19<sup>+</sup> cells, respectively. At the last available control (median follow-up of 11 months; 1-28), 8/9 patients had still not recovered the baseline CD19<sup>+</sup> cell count (median value: 6×10<sup>3</sup>/L; 0-295). The following side effects were observed: 3 cases of papulosquamous dermatitis, 1 case of fever and 1 case of fever and demantritis. Conclusions. Five/11 (45%) ITP patients had an early response to Rituximab (1 CR, 3 PR, 1 MR), that persisted in 3 cases. No late responses were observed. The response was independent from the post-therapy CD19<sup>+</sup> cell. No serious infections were observed during the clinical follow-up. No patient had to stop therapy because of severe side effects.

**0933**

**ISOLATED THROMBOCYTOPENIAS: ‘NATURAL’ HISTORY OF 281 CASES**

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**Aims.** Evaluate the outcome of isolated thrombocytopenias (patients without any hematological disorder other than thrombocytopenia and without any known underlying disease possible cause of thrombocytopenia), the probability of hemorrhagic events and need of therapy. Methods. A series of 281 patients with isolated thrombocytopenias - 105 men (37%) and 176 women (63%) were followed up as outpatients for range of 1-310 months (median 23 - mean 44). The platelet count (PLT) at diagnosis ranged from 1000 to 137000/mm<sup>3</sup> (median 89000 - mean 78000). The clinical classification at diagnosis was: 25% (95% C.I.: 20.0 - 30.4) severe - PLT under 50000/mm<sup>3</sup> - 56% (C.I.: 30.3 - 41.9) moderate - 51000-100000/mm<sup>3</sup> - 39% (C.I.: 33.1 - 44.8) mild - 10000-150000/mm<sup>3</sup>; at last follow up these percentages changed to: 10% severe - 29% moderate - 33% mild and 28% normal (>150000/mm<sup>3</sup>). Pseudotrabocytopenias resulted in 51 patients (11% - C.I.: 7.6-15.5; 9 men (8.6%) and 22 women (10.0%); p=0.412 - N.S.). The mean platelet life span was 7.5 days (C.I.: 5.1 - 9.9). Blood and urinary tract hemorrhages were the most frequent symptoms: 8 (4.8%) nosebleeds requiring nasal packing, 3 (1.8%) increased menstrual bleeding and 1 (0.6%) macrothrombocytopenia. No patient suffered an ICH or severe bleeding requiring transfusion. The mean platelet count on admission was 21,4×10<sup>3</sup>/L, lowest count 3×10<sup>3</sup>/L. Bone marrow aspiration was performed almost in all cases. Initial management consisted of no drug treatment in 9 patients (5.6%), intravenous immunoglobulins (IVIG) in 17 (10.6%) and glucocorticosteroids (GS) in 134 (83.8%). IVIG were used just in infants as they were expensive form of treatment. All patients improved regardless of the management strategy used. The mean length of stay in hospital in the period between 1998-2001 was 14,5 days, and between 2002-2005 7,9 days. We consid-
AUTOIMMUNE THROMBOCYTOPENIA: CLINICAL AND HEMATOLOGICAL OUTCOME OF PATIENTS AFTER DANAZOL ADMINISTRATION AS FIRST LINE OR REFRACTORY DISEASE TREATMENT

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Introduction. First line treatment of Autoimmune thrombocytopenia (AT) consists of corticosteroids and intravenous immunoglobulin. In refractory cases, splenectomy is indicated. In older patients or in those with a worse performance status, long term corticosteroid administration and splenectomy may be harmful and alternative treatments are under evaluation. Danazol is an androgen with mild, rare and reversible adverse events, which plays a role in AT therapy. Aim of the study. The aim of our study was to estimate the effectiveness and safety of danazol treatment in newly diagnosed or relapsed patients with AT. Patients and Methods. 35 patients (25 male/12 female) suffering from AT (30:ITP, 4:MDS/ITP, 1:low grade-NHL/ITP) were studied from 2002 to date. Median age was 65 years (23-92 years). 21 patients were >60yo and newly diagnosed, while 14 patients were <60 years and relapsed. Patients over 60 years with newly-diagnosed AT, no severe bleeding and Plts>20000/µL were treated with Danazol alone (200 mg q8h p.o. daily). The same-aged patients with Plts<20000/µL or/and with severe bleeding were initially treated with an induction regimen (IR) (dexamethasone 40 mg/day x 4days iv/or and IVIG 0.4g/kgBW/day x 5days iv) aiming at rapid platelet count restoration, because of slow response to danazol per se. Patients below 60 years with relapsed AT after standard treatment, were treated with Danazol alone (200 mg q8h p.o. daily). Patients who relapsed under danazol treatment, were treated with IR again or low dose methylprednisolone (4-5 mg per os daily) was added to danazol. After second rapid platelet count restoration, patients continued danazol monotherapy. First and second response to danazol was separately estimated in those patients. Average follow up period was 19 months, median was 7 months. Response criteria were defined as follows: Clinical remission: absence of bleeding manifestation; complete hematological remission (CR): Plts>140000/µL; Partial hematological remission (PR): Plts 50000-139999/µL; minimal hematological remission (MR): Plts 20000-50000/µL; no hematological remission (NR): Plts<20000/µL. Results. The overall response rate in patients treated with danazol alone (23/35) was 56.5% (CR 39.1%, PR 17.4%).

In patients over 60yo treated with primary IR and continued with danazol alone (12/35) the response rate was 58.3% (CR 38.5%, PR 20.8%). In patients with interim response to danazol—either alone or after initial IR administration- 40% (14/35) relapsed during follow up. These patients were treated with a second IR and returned to danazol monotherapy. 86% of them achieved second response to danazol (CR 42%, PR 42%) (Table 1). Co-administration of low dose methylprednisolone induced the response rate to danazol even in patients with standard corticosteroid adverse events. Average duration of response to danazol was 19 months, median was 7 months. The median time to response to danazol was 1 month. 97% (34/35) of patients showed clinical response and 31.4% (11/35) had adverse events due to danazol treatment; 9/35 had elevated liver enzymes (2/9 drug cessation due to severe reversible transaminasemia), 2/35 had mild renal function impairment. Discussion. Danazol is a safe and cheap treatment for autoimmune thrombocytopenia, as second line therapy in young relapsing patients, or as first line treatment in older patients, because long-term corticosteroid treatment is avoided with this schedule.

Table 1.

<table>
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<tr>
<th>Initial Treatment Options</th>
<th>CR</th>
<th>PR</th>
<th>MR</th>
<th>NR</th>
<th>NA</th>
<th>SUM</th>
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<td>4</td>
<td>3</td>
<td>–</td>
<td>–</td>
<td>7</td>
<td>19</td>
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<td>&gt;60 years, first diagnosed</td>
<td>2</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Danazol alone</td>
<td>2</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td>&lt;60 years, Danazol alone relapsed*</td>
<td>3</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>5</td>
<td>13</td>
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<tr>
<td>SUM</td>
<td>19</td>
<td>13</td>
<td>2</td>
<td>1</td>
<td>35</td>
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</table>

*:after standard initial treatment; **: IR: induction regimen (see text).

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DESCRIPTIVE EPIDEMIOLOGY OF IMMUNE THROMBOCYTOPENIC PURPURA IN THREE EUROPEAN COUNTRIES

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Backgrounds. The incidence and prevalence of immune thrombocytopenic purpura (ITP) has not been well characterized to date. Aims. To characterize ITP incidence and prevalence in three European countries overall and according to sex. Also, to determine whether ITP incidence and prevalence rates are increasing. Methods. Incident and prevalent cases were identified from databases from three countries: United Kingdom (General Practitioners Research Database (GPRD) 1990 through 2000), Germany (IMS Disease Analyzer Mediplus 1994 through 2008), and Netherlands (PHARMO database 1991-2005). GPRD and IMS include general practice physicians chosen such that their patients are representative of their respective countries. IMS also includes specialists. PHARMO contains hospitalization data from the National Medical Registry covering all hospital admissions in the Netherlands. Dutch population counts were obtained from Statistics Netherlands. ITP diagnoses were identified using the relevant coding system/codes for each database: ICD-9 287.3 (PHARMO), ICD-10 D693 (IMS Disease Analyzer Germany), and Read and OXMIS codes corresponding to an ITP diagnosis (GPRD). Incidence rates include only first time diagnoses, whereas prevalence rates include new and recurrent diagnoses. Results. The average annual incidence rate in the UK was 3.0 per 100,000 person years (95% confidence interval (CI) 2.7 to 3.3). Rates were fairly stable over time, ranging from 1.0 per 100,000 person years in 1998 to 3.7 per 100,000 in 1991. Average incidence, unadjusted for age, was 3.4 per 100,000 person years for women and 2.5 per 100,000 for men. Prevalence rates ranged from a low of 2.1 per 100,000 person years in 1990 and 2000 to a high of 8.1 per 100,000 in 1997. For Germany, average annual incidence was 2.7 per 100,000 person years (95% CI 1.7 to 4.1), ranging from 1.6 per 100,000 in 1996 to 5.1 per 100,000 in 1994. Incidence rates were comparable for German men and women. Prevalence ranged from 2.8 per 100,000 person years in 1999 to 7.3 per 100,000 in 1994. In the Netherlands, average annual incidence was 1.9 per 100,000 person years (95% CI 1.8, 2.0) varying little from 1991 through 2003 (1.7 to 2.1 per 100,000 person years). Average incidence was slightly higher for women than for men (2.2 per 100,000 and 1.8 per 100,000 person years, respectively). Annual prevalence ranged from 1.9 per 100,000 to 2.4 per 100,000 person years. Summary/Conclusions. ITP incidence and prevalence rates were less than 5 and 10 per 100,000 person years, respectively, in three major European countries. Incidence rates were higher for women than men in the UK and the Netherlands, but not in Germany. Rates did not increase during the period 1990 through 2003. These analyses of general practice and national medical databases provide a robust picture of recent ITP incidence and prevalence with a degree of precision lacking in previous evaluations of this relatively rare condition.
Physicians face therapeutic dilemmas when patients become resistant to known treatment in life-threatening conditions. A review of the literature shows a lack of comprehensive information on the clinical use of Cyclosporin A in the treatment of idiopathic thrombocytopenic purpura (ITP). Aims. To verify the usefulness of Cyclosporin A therapy in refractory ITP. Method. Study was carried out on long-term treatment (median 36 months) with Cyclosporin A (3 mg/Kg/day) in 14 selected adult patients with chronic, severe ITP resistant to all the usual therapies. Results. A median follow-up of 26.2 months shows that Cyclosporin A treatment obtained an improvement in 10 out of 14 patients (71%); 9 patients (64%) achieved a complete response and one (7%) a partial response. Four patients (29%) were unresponsive. In 5 patients (50%) the discontinuation of Cyclosporin A was successful, maintaining the remission over time, without further therapy. In the remaining responsive patients, the remission was dependent on continued drug. The Cyclosporin A intolerance was slight in spite of long-term treatment and no nephrotoxicity occurred. Conclusions. Our study shows the safety and efficacy of Cyclosporin A therapy in resistant ITP. Because the potential role in second neoplasia appearance and the well known teratogenic role of this immunosuppressors, cyclosporin A will be done only in resistant ITP cases (dramatic clinical cases).

Myelodysplastic syndromes II

THE SALVAGE TREATMENT WITH CYCLOSPORIN A IN IDIOPATHIC THROMBOCYTOPENIC PURPURA

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Backgrounds. Physicians face therapeutic dilemas when patients become resistant to known treatment in life-threatening conditions. A review of the literature shows a lack of comprehensive information on the clinical use of Cyclosporin A in the treatment of idiopathic thrombocytopenic purpura (ITP). Aims. To verify the usefulness of Cyclosporin A therapy in refractory ITP. Method. Study was carried out on long-term treatment (median 36 months) with Cyclosporin A (3 mg/Kg/day) in 14 selected adult patients with chronic, severe ITP resistant to all the usual therapies. Results. A median follow-up of 26.2 months shows that Cyclosporin A treatment obtained an improvement in 10 out of 14 patients (71%); 9 patients (64%) achieved a complete response and one (7%) a partial response. Four patients (29%) were unresponsive. In 5 patients (50%) the discontinuation of Cyclosporin A was successful, maintaining the remission over time, without further therapy. In the remaining responsive patients, the remission was dependent on continued drug. The Cyclosporin A intolerance was slight in spite of long-term treatment and no nephrotoxicity occurred. Conclusions. Our study shows the safety and efficacy of Cyclosporin A therapy in resistant ITP. Because the potential role in second neoplasia appearance and the well known teratogenic role of this immunosuppressors, cyclosporin A will be done only in resistant ITP cases (dramatic clinical cases).

RESULTS OF CLONALITY ASSAY AND MEASUREMENT OF APOPTOTIC RATE AND TELOMERE LENGTH SUPPORT USEFULNESS OF SEPARATION OF REFRACTORY CYTOPENIA FROM REFRACTORY ANEMIA AS A DISTINCT SUBTYPE OF EARLY MDS

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Background and aim of the study. The degree of clonality, telomere length...
and the rate of apoptosis represent laboratory markers that may be relat-
ed to the progression of pathological clone in patients with myelodys-
plastic syndrome (MDS). In this study we investigated these markers in
dent patients with different subtypes of MDS. Methods. X-chromosome
inactivation pattern clonality assay based on PCR amplification of poly-
morphic short tandem repeats of the human androgen receptor (HUMARA)
gene was performed in granulocytes, CD14+ and CD34+ cell sub-
populations isolated from bone marrow and peripheral blood of 58
females with primary MDS and 20 healthy controls. The results were
compared with measurements of the telomere length by Terminal Repeat
Fragmentation method (TRF) and with apoptotic rate of CD34+ and GlyA+
subpopulations assessed by flow cytometry (Annexin V and TUNEL
method). Results. In 19 patients with advanced MDS (RAEB, RAEB-
T, CMML, monge/thrombocytosis, or the FAB classification) clonal
CD14+ cell subpopulations (allele ratio ≥9:1) were present in bone mar-
row, peripheral blood of 74% and 87% of patients, respectively.
Shortened telomere length (TRF < 7.5 kb) and low rate of apoptosis of
CD34+ bone marrow cell subpopulation was present in all patients with
advanced MDS. In patients with early MDS, clonal patterns of hemoapoiesis were present only in 2 out of 17 patients (12%) with RA,
RARS or 5q- syndrome according to the WHO classification. On the
other hand, clonal granulocyte or CD14+ cell subpopulations were pres-
ent in bone marrow or peripheral blood of 20 out of 22 patients (90%)
with RCDM according to WHO criteria. In accordance with these
results, nuclear factor-kappaB (NF-κB) was increased in 19 patients with early MDS and 31% in those with clonal granulocyte or CD14+
subpopulations exhibited low apoptotic rate of CD34+ bone marrow cells
(5-12%). On the contrary, 80% of patients with non-clonal cells had
increased apoptotic rate of CD34+ cells (30-80%). Reduced telomere
length was found in 71% patients with clonal subcell populations v.s.
45% in those with non-clonal cells. Median survival of patients with
early MDS and clonal cells was 62.5 months vs. 47.8 months in those
with non-clonal cells (p<0.05) and 65.7 months in RA patients v.s.
50.0 months in RCDM patients (p<0.05). Conclusions. The results confirm
our preliminary observations suggesting that RCDM represents a sepa-
rate clinical and laboratory entity with adverse prognosis which is dis-
tinct from RA and support hypothesis of multistep pathogenesis of
MDS, where dysplasia limited to erythropoiesis may represent an early
step and multilineage dysplasia is a subsequent step reflecting pro-
gression of pathological clone.
Funding: The study was supported by scientific program MZO 00023736
from Czech Ministry of Health.

0940 INHIBITION OF THE MKK3-P38 MITOGEN-ACTIVATED PROTEIN KINASE PATHWAY IS REQUIRED FOR NEUTROPHIL DIFFERENTIATION OF HUMAN CORD BLOOD DERIVED CD34+ CELLS
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Patients with myelodysplastic syndromes (MDS) suffer from recur-
brent bacterial infections as a result of differentiation defects of the neu-
triphil lineage. While limited number of genetic defects of MDS progen-
itor cells has been described, the defective intracellular signal transduc-
tion pathways modulating these developmental defects remain unde-
flled. Mitogen-activated protein (MAP) kinase cascades play a key role in
regulating a plethora of cellular processes. They typically are organ-
ized in a three-kinase architecture consisting of a MAPK, MAPK activa-
tor (MKK) or MAPK kinase, and a MKK activator (MAPK kinase kinase).
The p38 MAPK pathway mediates a wide variety of cellular processes in
response to extracellular stimuli such as UV light, osmotic shock,
inflammatory cytokines and growth factors and it has been shown that
MKK3 and MKK6 are the main MKKs activating p38. Although p38
has been demonstrated to regulate differentiation in several cell types, its role
in regulating neutrophil development in both normal as well as in defec-
tive MDS granulopoiesis remains to be investigated. Aims. The aim of
this study was to investigate the role of the p38 MAPK signalling mod-
ule in neutrophil differentiation and to determine whether p38 MAPK
signalling may play a role in aberrant neutrophil development in MDS
Mononuclear cells were isolated from umbilical cord blood using a ficoll-
paque solution and MACS immunomagnetic cell separation was used to
isolate CD34+ cells. Cells were cultured in IMDM supplemented with
9% serum and differentiation towards neutrophils was induced upon
LPS, IL-1, GM-CSF, G-CSF or GM-CSF plus IL-3, each of which
inhibitors only partially block apoptosis (25-41%
nomenamide and 3-aminobenzamide) only partially block apoptosis (25-41%)
over, inhibitors of the poly (ADP-ribose)polymerase, PARP , (nicoti-
name-3 (DEVD-fmk), -2 (VDVAD-fmk), -8 (IETD-fmk), -9 (LEHD-fmk)
and pan-caspase inhibitor (ZVAD-fmk) did not block apoptosis. More-
over, inhibitors of the poly (ADP-ribose)polymerase, PARP, (nicoti-
namide and 3-aminobenzamide) only partially block apoptosis (25-41%)
and 51-45% decrease, respectively). Conclusion. 5-azacitidine activates
PARP, which in turn induces mitochondrial dysfunction. At the mito-
chondrial level this compound suppresses anti-apoptotic properties
(cleavage of Bcl-2) and increases pro-apoptotic activities (cleavage of
Bax and Bid). These events result in the loss of mitochondrial mem-
brane potential and release of cytochrome-c into the cytosol. As PARP
inhibitors only partially block loss of mitochondrial membrane potent-
ial, and caspase inhibitors did not have any effect on any of apoptosis
manifestations, we conclude that 5-azacitidine induces cell death via
activation of caspase-independent pathway. It seems that caspase
activation plays a secondary role in this process.

0941 MITOCHONDRIAL INVOLVEMENT IN 5-AZACYTIDINE-INDUCED APOPTOSIS
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Backgrounds. Although 5-azacitidine is the only drug approved by the
FDA for high-risk MDS, its mode of action has not been well character-
ized. Aim: The aim of the study was to investigate the mechanisms of
5-azacitidine-induced cytotoxicity in myeloid FS9 cells in order to opti-
mize the use of this drug. Methods. Cells were incubated with 0.1-2 µM
5-azacitidine for 4-48 hours. Nuclear and cytoplasmic p38 phosphory-
lar staining of lactoferrin.

0942 CLINICAL CHARACTERISTICS AND TREATMENT OF 217 NEW MDS PATIENTS DURING THE YEAR 2005 IN A TERTIARY REFERRAL CENTER
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H.K. Habersang,1 F.F. Fox,1 A.M. Aivado,1 C.A. Andresen,1
G.K. Kobbe,1 B. Hildebrandt,1 N. Gattermann,1 R. Haas1
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Backgrounds. Heterogeneity of myelodysplastic syndromes not only
relates to the biology of the disease but also to the spectrum of patients
seen by different health care providers. Aims. We gathered data on patient characteristics and treatment of 217 new MDS patients seen in our medical center during the year 2005. Methods. All MDS patients treated either in the hematological outpatient clinic or on the wards were documented. A diagnosis of MDS was made according to the standards of the German MDS Registry in Düsseldorf, including central morphologic assessment. Patients were followed for any kind of complication, disease progression, and therapeutic intervention during the year 2005. Results. In 2005, a total of 217 patients were seen at our institution. In 90 patients (41%) the diagnosis of MDS was made during that year, either before or after referral. 95% had primary MDS, 7% were diagnosed as treatment-related MDS. The distribution among MDS types was: 10 RA, 4 RAEB, 64 RARS, 26 RCMD-RS, 26 RAEB I, 33 RAEB II, 25 CMML, 6 patients with 5q- syndrome, and 23 patients with RAEB-T. A karyotype analysis was available in 82% of patients (n=179). 100 patients (56%) pre-sented with a normal karyotype. According to the International Prognostic Scoring System (IPSS), 25% of the patients belonged to the low-risk, 36% to the Intermediate-1, 24% to the Intermediate 2, and 15% to the high-risk group. A 5q- anomaly. Either as sole abnormality or as part of a more extensive derangement, was found in 17 patients. There were 121 males and 96 females. 123 (59%) patients were treated in our outpatient clinic only; with a median number of 4 consultations with the doctor (1-6). 91 (41%) pa-tients were admitted to the hospital, 68 of whom (31%) were treated on the ward as well as in the outpatient clinic. Reasons for hospitalization were disease complications like infec-tions, hemorrhages, and bad general condition in 55%. In 45% of cases, patients were admitted for intensive chemotherapy, allogeneic stem cell transplantation, or any kind of treatment that requires inpatient care, including certain clinical trials. The median number of hospitalizations per patient was 1 (1-6). 29 patients (13%) died during the year 2005, 59 patients (27%) showed progression to AML. 81% of patients received at least one unit of packed red cells. Summary: With regard to MDS sub-type distribution, patients seen in our institution did not differ much from the MDS population as a whole. Still, a referral bias is present, reflected by a large proportion of patients requiring inpatient care, either for management of MDS-related complications or intensive treatment of the underlying bone marrow disease.

**0943**

**ACQUIRED α-THALASSEMIA IN MDS (AT-MDS): RARE MUTATIONS DETECTED IN TWO FEMALES**

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Background. In contrast to the classical thalassemias, two distinct thalassemias were recently described in which the molecular defect does not reside in the globin genes but in a transcriptional activator of α-globin genes. This protein, dubbed ATRX, is mutated in the rare inherited disease of α-thalassemia (AT) with mental retardation (AT-RX syndrome) whose affected individuals show a mild form of AT. In addition, and independent of the ATR-X syndrome, there have been approximately 100 case reports worldwide of the association of an acquired form of AT with hematological neoplasms, the large majority of those cases being MDS (AT-MDS). The clinical characteristics of such patients encompass the typical features of the underlying hematological disorder plus microcytic anemia. The latter is due to massively reduced α-globin gene tran-scription resulting in excess hemoglobin H (HbH), as revealed by supravital staining of peripheral blood erythrocytes and hemoglobin electrophoresis. The molecular defect of AT-MDS lies in a mutation of the ATRX protein and, thus, represents a loss of α-globin-thalassemia. AT-MDS shows a striking male preponderance. Methods. Supravital staining, Southern blotting, and DNA sequencing by denaturing high-per-formance liquid chromatography. Results. Here we report on two female pts with AT-MDS. The first was a 69 year old pt who was diagnosed with MDS-RARS/MSD over 1 year ago (JAK2 negative) with normal karyotype. She presented with microcytic anemia (Hb 7.5 g/dL, MCV 76.5 fl, HbE 22.4 pg) and increasing thrombocytosis (953.000/µL maximum). Supravital staining of a peripheral blood smear revealed erythrocytic HbH inclusions. Sequencing of the ATRX coding sequence revealed a novel missense mutation with an Asp835 substitution in codon D225 (p=0.0001). Results. A karyotype analysis in 6 patients (D225Asp) resulted in more than 2 forms of triplicated acid substitution in exon 32. It represents the 14th ATRX mutation described thus far and, moreover, the first mutation detected in a female. The second pt (61 years old) with initially RA, normal karyotype (JAK2 negative) and microcytic anemia (Hb 10.4 g/dL, MCV 69 fl, HbE 15.2 pg) had increasing erythrocytosis of ±93 Mio/µL maximum. The genetic analysis showed a ATRX point mutation in exon 8 (G521A) which results in an amino acid change from cysteine to tyrosine and consequently in a loss zinc finger. Conclusions. Though AT-MDS is mostly diagnosed in males we have diagnosed two females, both showing ATRX mutations. Microcytic anemia in association with a hematological neoplasm, most commonly MDS, should alert to AT-RX syndrome. Molecular mechanisms by which mutations cause acquired α-thalassemia probably include epigenetic alter-ations of DNA methylation and chromatin structure. The remarkable thronboctyosis and erythrocytosis, respectively, in our 2 pts are at least suggestive of other phenotypic abnormalities possibly associated with the acquired ATRX genotypes on the MDS background.

**0944**

**SERIAL DETERMINATION OF FLT3 MUTATIONS IN MDS PATIENTS AT DIAGNOSIS, FOLLOW UP, OR AML TRANSFORMATION: FLT3 ITD/ASPS33 MUTATIONS INCIDENCE AND THEIR PROGNOSTIC SIGNIFICANCE**


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Background. The genetic/molecular alterations that lead to MDS are not fully elucidated. MDS can be considered as pre-leukemia but the precise genetic/molecular events occurring during transition to AML are unknown. Aim. The aim of this study was a) to investigate the incidence of FLT3 mutations (ITD/Asp835) in MDS patients at the time of MDS diagnosis and during disease evolution, b) to analyze if the presence of FLT3 mutations correlates to AML transformation and c) to investigate the prognostic significance of FLT3 mutations in MDS patients. Methods. Genomic DNA was extracted from bone marrow aspirate samples from 97 patients with MDS (RAEB-t and therapy-related MDS were excluded). All patients had bone marrow aspirate at presentation and at sever-al time points during their follow up (2-10 samples per patient). Patient DNA was amplified by PCR with specific primers for the detection of FLT3 internal tandem duplication (ITD). ITD positive samples (PCR bands >529bp) were cloned and sequenced. Asp 835 point mutations in exon 20 of the FLT3 gene were detected with PCR followed by digestion with EcoRV of the 195 bp PCR product. Non fully digested products (mutated) were cloned and sequenced (10 plasmids/patient) to verify the existence of Asp 835 mutation. Fisher’s exact test and unpaired t-test were used for statistical analysis. Survival curves comparison was done by the log-rank test. For all analyses the p-values <0.05 were considered statistically significant. Results. Three of the 97 patients had FLT3 mutations at presentation: one patient with both ITD and Asp835 (RAEB-BM blasts 16%), one patient with ITD only (RAEB) and one patient with Asp835 only (RAEB). Forty two patients progressed to AML including the three patients that carried FLT3 mutations at MDS diagnosis. The total incidence of FLT3 mutations at the time of AML progression was 14.3% (6 out of 42), with 3 additional patients acquiring FLT3 mutation at the time of progression. In these 3 latter patients, FLT3 mutations were detectable in bone marrow samples 4-6 weeks before overt leukemic transformation. All identified FLT3 mutations were in frame as shown by sequence analysis and suggest a gain of func-tional mutational event. Patients with FLT3 mutations had 4.5 times high-er risk of transformation to AML compared to patients without muta-tions (Cox’s model application). Survival curves comparison by long-rank test showed a statistical significant difference between MDS patients with FLT3 mutations compared with those without mutations (p=0.0001), as well as between transformed MDS patients with FLT3 mutations compared with transformed MDS patients without muta-tions (p=0.01). Two extra patients acquired FLT3 mutations 12 and 32 months respectively after MDS diagnosis; both these patients died 2 and 6 months respectively, after FLT3 mutation detection, from infection, before evolution to AML. Conclusion. Our study shows that FLT3 mutations seem to be the critical additional genetic event that transforms a minority of MDS to AML; these mutations can be detected before trans-formation to AML and effective FLT3 inhibitors, when available, might be a potent therapeutic modality for these patients.
ABNORMAL PERIPHERAL BLOOD PROGENITORS ARE CONSISTENTLY OBSERVED AFTER CELL CULTURE IN MYELODYSPLASTIC SYNDROME (MDS), ALLOWING THE DIAGNOSIS OF EARLY STAGE OF THE DISORDER

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Background. The diagnosis of MDS may be difficult if dysmyelopoietic and cytogenetic features are absent or inconspicuous, and in many instances follow up with repeated investigations must be achieved to confirm or to rule out the diagnosis. To ascertain whether abnormal progenitors are already present in peripheral blood from MDS patients, and to improve the diagnosis of early steps of the disease. Methods. We studied in vitro peripheral blood progenitor cell growth from different groups: healthy patients (T)(n=9), MDS (all WHO subtypes)(n=24), non malignant secondary cytopenia(s) (C)(n=9), and one group displaying cytopenia(s) of uncertain diagnosis (n=15). The latter group had follow up for up to 2 years. After percoll (1.077g/mL) separation, blood mononuclear cells (MNC) were collected, CD34+ percentage was determined using flow cytometry, and a number of cells equivalent to 200 CD34+/ml was seeded in semi-solid collagen gel (stem 0.48 medium; Stem Alpha, France). After 14 days, collagen gels were transferred to glass slides and stained with MG C to look for colonies (growth number and morphology). Results. Median number of colonies (CFU-GM and BFU-E) for 105 MNC was found similar for T (11 and 40) and C (8 and 27), but lower for MDS patients (5 and 5) (p<0.004). Median clonogenic efficiency of CD34+ cells was 5 times lower for MDS patients, but in comparison to non malignant secondary cytopenias (p<0.001). Morphologic analysis of colonies from collagen gels allowed estimating cellular degeneration: the ratio viable colonies/ all colonies (v/a), for both CFU-GM and BFU-E, was always >0.60 in T and C groups, whereas it was always <0.56 in MDS patients (p<0.003). Among the 13 patients displaying cytopenias of uncertain diagnosis, three had a CFU-GM and/or BFU-E v/a ratio<0.58, and 10 a v/a ratio >0.60. The three patients with an abnormal v/a ratio evolved to a MDS (RAEB or RCMD) within 2 years following cell culture. Nine patients with a normal v/a ratio recovered a normal cell count within 6 months (n=6) or developed progressive kidney failure (n=3). One patient with normal v/a ratio remained unclassified after 9 months of follow up. Conclusions. Whatever the MDS subtype, cell culture demonstrated that abnormal progenitors were consistently found within peripheral blood, which demonstrated limited in vitro growth and a high degeneration rate. As a high degeneration rate of progenitors was not observed in non malignant disorders, study of peripheral blood progenitors in patients of unknown origin is proposed as a diagnostic tool to ascertain or to rule out diagnosis of early MDS.

INCIDENCE OF MDS WITH 5q- KARYOTYPE ANOMALIES

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Backgrounds. In a previous study (Germing et al., Haematologica, 2006) we found an overall incidence of myelodysplastic syndromes (MDS) of ~5/100.000 per year in the town district of Düsseldorf. Incidence figures were strongly age-related, with a significant rise after the age of 60, particularly in males. We calculated that approximately 4100 new cases of MDS are diagnosed in Germany each year. In individuals below the age of 40 years, the incidence of MDS is only ~0.4/100.000 per year. Aim: The purpose of the present study was to assess the incidence of MDS with cytogenetic aberrations involving 5q-. Methods: We looked into the German MDS Registry in Düsseldorf for cytogenetic findings and related them to incidence figures calculated for the town district of Düsseldorf. Results. The MDS Registry now comprises 2897 patients, including 2734 with primary MDS (94.4%) and 162 with secondary MDS (5.6%). Median age at the time of diagnosis was 72 years, ranging from 16 to 96. Only 4% of the patients were younger than 40, and 8% younger than 50. There were 1578 males (54.4%) and 1319 females (45.5%). The distribution among IPSS risk groups is as follows: 20% low, 31% intermediate-1, 21% intermediate-2, and 28% high-risk. Notably, the low/int-1 group on the one hand, and the int-2/high risk group on the other hand, each have an incidence of about 2.5/100.000 per year, which is similar to that of acute myeloid leukaemia. In 1038 patients (36%), chromosomal analyses were performed at the time of diagnosis. Progenitors were karyotyped significantly younger (median: 64 years) than the MDS patient population as a whole (p=0.0065). Among those with a karyotype available, 180 patients (17%) had a 5q- anomaly, either as a single aberration (n=114) or together with one more chromosomal abnormality (n=12), or as part of a complex karyotype (n=53). This implies an incidence of MDS with 5q- anomalies of about 0.60/100.000 per year, equivalent to nearly 700 newly diagnosed cases per year in Germany. Among patients with a 5q- abnormality in our database, we identified only 21 females younger than 50 yrs and 7 females younger than 40. For males, the figures were similar. To summarize, MDS is one of the most frequent haematological disorders, particularly in the elderly. Patients with karyotype anomalies involving 5q- represent about 17% of MDS patients with an estimated incidence of about 0.85/100.000 per year. Such patients are rare among individuals less than 50 years old. Because of the ‘greying of the population’ in developed countries, the number of all MDS patients, including those with a 5q- anomaly, is expected to rise.

ALTERATIONS IN THE NATURAL KILLER CELL RECEPTOR REPERTOIRE IN MYELODYSPLASTIC SYNDROMES

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Backgrounds. Myelodysplastic Syndromes (MDS) constitute a group of clonal stem cell disorders characterized by ineffective hematopoiesis and pancytopenia. In one third of the MDS patients the disease progresses to acute myeloid leukaemia. The only curative treatment for MDS is allogeneic stem cell transplantation (SCT). Several studies have shown that natural killer (NK) cells play an important role in the outcome of SCT in acute myeloid leukaemia patients. These results suggest that NK cell activity may constitute an important therapeutic tool in the treatment of hematological diseases such as MDS. So far, the role of NK cells in the pathogenesis of MDS is poorly understood. Aim: The aim of this study was to investigate the NK cell receptor repertoire in patients with MDS. Methods. Bone marrow (BM) and peripheral blood (PB) was collected from patients with MDS and NK cells were analyzed for their receptor repertoire using multi-color flow cytometry. Results. MDS patients displayed severe alterations in their NK cell receptor repertoire with decreased expression of several activating NK receptors, including DNAM-1, NKG2D, and CD16. These alterations were confined to BM and not to PB. One patient had abnormally high levels of CD56dim NK cells displaying a reversed ratio between CD56dim and CD56bright NK cells with 75% and 50% regulatory CD56dim NK cells in BM and PB, respectively. Conclusions. Our preliminary results show that MDS patients display several phenotypic aberrations in their NK cell repertoire. This may have functional consequences and influence pathogenesis and response to immunomodulatory treatments for MDS. Uncovering a role for NK cells in the recognition of MDS tumor cells may set the stage for future NK cell-based immune therapies against MDS.

MYELOID ANTIGEN EXPRESSION ON CD34 POSITIVE BLASTS IN MYELODYSPLASTIC SYNDROMES

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Backgrounds. The myelodysplastic syndromes (MDS) constitute a heterogeneous group of clonal hematopoietic stem cell disorders. They are characterized by abnormal bone marrow (BM) differentiation, peripheral blood cytopenias and a risk of transformation into acute myeloid leukaemia (AML). The diagnosis of MDS depends on morphological criteria and cytogenetics and is sometimes difficult to make and subjective. Aim: In this study we evaluated the potential of immunophenotyping CD34+ hematopoietic precursors for the diagnosis and classification of myelodysplastic syndromes. Methods. Bone marrow samples of patients with different forms of MDS (51 samples), of healthy controls (15 samples), of patients with cytopenia not due to MDS (disease controls; 39 samples) and of patients with AML (25 samples) were examined. MDS
and AML samples were classified according to the WHO criteria. The expression of CD19, CD10, CD13, CD15, CD33, CD117 and CD45 antigens was detected on the CD34+ cells by flow cytometry. Statistical analysis was done with a Mann-Whitney test. Results. The number and immunophenotype of the CD34+ cells in BM of disease controls was similar to that in normal bone marrow. Only the number of CD34+ CD13+ blasts was lower. A high number of CD34+ cells was found in MDS and AML. This number correlated with the percentage of blasts found by cytomorphology. The increase of the CD34+ cell number was accompanied by the increase of the myeloid precursors (CD34+ CD117+) and a decrease of the B cell precursors (CD34+ CD19+). CD117 appeared to be the best marker for myeloid precursors, followed by CD15, especially when the number of blasts was high. A wide range of CD34+ CD13+ and of CD34+ CD33+ positive cells was found in all types of samples. CD133 expression was increased in MDS samples with excess of blasts and in AML. No statistical difference was found between the different groups for the CD33 expression. The myeloid antigen expression on CD34+ cells in refractory anemia (RA), refractory anemia with ringed sideroblasts (RARS) and refractory cytopenia with multilineage dysplasia (RCMD) was comparable, although a low positivity for CD133 was found in RARS patients. In MDS with excess of blasts, no statistically different values were found between the CD34 antigen expression on the CD34 cells of the two subtypes (RAEB-1 and RAEB-2). The phenotype of blasts in AML patients with blasts (previously RAEB-0) was comparable to that found in AML with multilineage dysplasia and other AML patients. Conclusion. MDS is characterized by a variable number of CD34+ cells in the bone marrow. This number correlated with the percentage of blasts found by cytomorphology. An increase of the number of CD34+ is accompanied by an increase of the myeloid precursors and a decrease of the B lymphoid precursors. In MDS samples with less than 5% blasts the myeloid antigen expression on the blasts was comparable to that in disease controls. In MDS samples with an excess of blasts the phenotype was closer to that of AML.

0949
LOW-RISK MYELODYSPLASTIC SYNDROMES FROM PIEMONT MDS REGISTRY: A COMPARATIVE REVIEW OF BONE MARROW ASPIRATE SMEARS AND BONE MARROW BIOPSY SPECIMENS

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Aims. to assess the contribution of bone marrow aspirate (BMA) and bone marrow biopsy (BMB) on diagnosis and prognosis of low-risk MDS. Patients and methods. We reviewed 82 cases of MDS with low marrow blasts (<5%), consecutively admitted in five hospital of Piedmont between 1998 and 2004. All patients were studied on admission, with full blood count, cytogenetics, BMA and BMB. Pts notes were recorded in the archives of Piedmont MDS Registry. Prerequisites for evaluation were: 1) no AML patients with blasts (previously RAEB-0) was comparable to that found in AML with multilineage dysplasia and other AML patients. Conclusion. MDS is characterized by a variable number of CD34+ cells in the bone marrow. This number correlated with the percentage of blasts found by cytomorphology. An increase of the number of CD34+ is accompanied by an increase of the myeloid precursors and a decrease of the B lymphoid precursors. In MDS samples with less than 5% blasts the myeloid antigen expression on the blasts was comparable to that in disease controls. In MDS samples with an excess of blasts the phenotype was closer to that of AML.

0950
OCURRENCE OF THE JAK2 V617F MUTATION IN PATIENTS WITH REFRACTORY ANAEMIA WITH RINGED SIDEROBLASTS ASSOCIATED WITH THROMBOCYTOPENIA (RARS-T)

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Backgrounds. The WHO classification establishes a new category, the Myelodysplastic/Myeloproliferative diseases (MDS/MPD). This category includes myeloid disorders that have both dysplastic and myeloproliferative features. MDS/MPD, U-refractory anemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T) is incorporated in this category as a provisional entity. The clinical and morphological features consist of the myelodysplastic syndrome, refractory anemia with ringed sideroblasts (RARS) but with a marked thrombocytosis (>600×10³/L). The megakaryocytes are enlarged in size. Essential Thrombocytemia (ET) is a Chronic Myeloproliferative Diseases (MPD). A single point mutation of JAK2 (Val617Phe) has been detected in half the patients with ET. Aims. The presence of the JAK2 mutation was assessed in a cohort of patients with RARS, including 3 cases with RARS-T. Methods. We obtained DNA from blood samples from 3 patients with RARS-T. These samples were analysed using the allele-specific PCR methodology described by Baxter et al.1 DNA samples from 16 RARS and from 14 ET patients were also sequenced. Results. In the three cases with RARS-T, the V617F mutation of the JAK2 gene was detected, but none of the other cases with RARS showed the mutation. Interestingly, endogenous erythroid colony formation in vitro was negative in two of them. Bone marrow exams showed hypercellularity with prominent megakaryocytic proliferation, enlarged in size. None of them showed the typical small-sized megakaryocytes of the 5q-syndrome. After a long follow-up (15 years) one case evolved to myelofibrosis. In the ET group, 13 out of 21 cases showed the JAK2 mutation. Conclusion. RARS-T appears to be the coexistence of two disorders, with erythropoiesis showing the characteristics of the RARS and megakaryocytes those of ET. Further data from other groups are necessary to confirm the prevalence of the JAK2 mutation in RARS-T.

References

0951
WT1 IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES: A USEFUL MOLECULAR MARKER FOR RISK ASSESSMENT

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Backgrounds. Myelodysplastic syndromes (MDS) are clonal hematopoietic stem-cell disorders characterized by ineffective dysplastic hematopoiesis involving one or more cell lineages and characterized by peripheral-blood cytopenias and a high risk of progression to acute leukemia (AML). According to WHO classification, MDS can be classified in these following groups: refractory anemia (RA), RA with ringed sideroblasts (RARS), RA with excess of blasts type I and II (RAEB I and II), refractory cytopenia with multilineage dysplasia (RC+Dys), del (5q) syndrome, and MDS unclassifiable (MDS unclass). The Wilms’ tumor gene (WT1) is a tumor suppressor gene coding for a zinc-finger transcription factor located on chromosome 11p13, which was originally identified for its involvement in the pathogenesis of the Wilms’ tumor. In normal peripheral blood (PB) and bone marrow (BM), WT1 expression is reported to be low and sometimes undetectable even by RT-PCR. By contrast, WT1 is highly expressed in most acute leukemias, and its level of expression is associated with the presence, persistence, or reappearance of leukemic hematopoiesis. Aims. WT1 gene expression could represent a useful marker in MDS to establish prognosis and progression of disease. Methods. BM samples from 36 MDS patients (16 RA, 7 RAEB I, 4 RAEB II, 4 RARS, 5 deletion of 5q, 2 MDS unclass) were tested for WT1 expression at diagnosis and after 6 months. WT1 gene expression

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was evaluated by methods of real-time quantitative PCR (RQ-PCR).

**Results.** At diagnosis, 21 BM samples (10 RA, 6 RAEB I, 4 RAEB II, 1 RARS, 1 MDS unclass) expressed WT1 transcript amounts greater than the ranges level. The degree of WT1 expression was highly correlated with the type of MDS, was much higher in RAEB I and II compared with RA, and other types, and increased during disease progression. Moreover, a significant correlation was found between WT1 expression levels, baseline hemoglobin levels, and the presence of cytogenetic abnormalities. The patients received only a supportive therapy if necessary. After 6 months, 7 patients (2 RA, 3 RAEB I, 2 RAEB II) converted to AML. All of these patients showed at diagnosis an high WT1 expression level and a further elevation of WT1 expression after 6 months. **Conclusion.** WT1 expression has been previously reported to be increased also in myelodysplastic syndromes. In this study, the data obtained show that in most MDS, including a large percentage of RA and almost the total number of RAEB I and II, WT1 is expressed above the range observed in normal controls in BM and that its expression is directly correlated with the type of MDS. In addition, even within each subgroup, a strong association is present between the level of WT1 expression and the blast percentage and the presence of cytogenetic alterations. The identification of a molecular marker so able to establish the tendency of MDS to progression can be of great help in decision making for MDS patients. In conclusion we believe that WT1 can be introduced as a additional marker to the standard parameters already considered in risk assessment for MDS.

**0952**

**COST-EFFECTIVENESS OF CHELATION THERAPY WITH DEFERASIROX VERSUS DEFEROXAMINE IN TRANSFUSION-DEPENDENT MYELODYSPLASTIC SYNDROME**

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**Background.** Patients with myelodysplastic syndrome (MDS) frequently receive chronic transfusions, along with chelation therapy to prevent complications of iron overload. Deferoxamine is an effective iron chelator, but must be administered as an 8-12 hour infusion 5-7 times per week, leading to poor compliance and/or quality of life. Deferasirox is a once-daily oral chelator that has been shown to produce reductions in liver iron concentrations and serum ferritin similar to those obtained with deferoxamine. **Aims.** To evaluate from a US perspective the cost-effectiveness of deferasirox versus deferoxamine in patients with transfusion-dependent MDS. **Methods.** Data from a variety of published and unpublished sources were used to estimate the cost-effectiveness of chelation therapy with deferasirox versus deferoxamine in MDS patients receiving frequent transfusions (28 per year). As there are no long-term studies describing the complications of iron overload in MDS, we focused on the short-term (i.e., one year) cost and quality-of-life effects of chelation therapy. As comparative data for deferasirox versus deferoxamine were unavailable, we estimated a relative dose of deferasirox based on results for MDS patients in a non-comparative Phase II study (20 mg/kg/d). **Conclusion.** The relative dose of deferoxamine that would result in similar efficacy (2.1) was based on data from comparative studies in other transfusion-dependent anemias. We conservatively assumed that patients would be fully compliant with chelation therapy. Cost-effectiveness was measured in terms of the ratio of the difference (deferasirox versus deferoxamine) in costs of chelation therapy to the difference in quality-adjusted life years (QALYs) over one year. Unit costs of deferoxamine and deferasirox were based on US wholesale acquisition costs. The cost of deferoxamine administration was based on actual transfusion charges, and the cost of deferasirox was estimated to result in a gain of 0.25 QALYs per patient. Utility data for MDS patients with transfusion-dependent anemias. Utilities for MDS patients receiving transfusions were based on published data for patients with anemia from metastatic cancer. The difference in quality of life for deferasirox versus deferoxamine was based on a study that used time-trade-off methods to estimate community-based preferences for oral versus infusional chelation. **Results.** One year of treatment with deferasirox is estimated to result in a gain of 0.25 QALYs versus deferoxamine (0.78 versus 0.55). If the price of branded deferoxamine is employed, total annual costs are estimated to be $1,427 greater with deferoxane versus deferasirox ($45,604 versus $44,177). The cost-effectiveness of deferasirox versus deferoxamine is $6,204 per QALY gained. If the price of generic deferoxamine is employed, costs are increased by $7,025 with deferasirox versus deferoxamine; the cost per QALY gained with deferasirox versus deferoxamine is $30,542. Cost-effectiveness of deferasirox versus deferoxamine was sensitive to the assumed dosages of deferasirox and defer-
**0954**
DISPARITIES IN CRITERIA FOR INITIATING CHELATION THERAPY FOR IRON OVERLOAD IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS)

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**Backgrounds.** The Myelodysplastic Syndromes Foundation, Inc., a non-profit organization established by an international group of physicians and researchers to provide an ongoing exchange of information relating to MDS, conducted an international survey of practices and treatments of clinicians with expertise in diagnosing and treating MDS patients. Survey responses relating to iron overload were studied because it is estimated that more than 40% of MDS patients require regular red blood cell transfusions. **Aims.** To analyze survey data on current expert clinical management strategies for iron overload in MDS. **Methods.** Descriptive statistics were used to analyze The MDS Foundation’s 2004-2005 Practices & Treatment Survey responses received by email and fax through August 2005. The survey, developed by hematologists with expertise in MDS, was distributed to the MDS Foundation’s Centers of Excellence (48 US and 53 European and other academic medical centers). **Results.** Of the MDS Foundation’s 102 Centers of Excellence, 70 (58 US and 32 European and other non-US centers) responded to the survey. Responses indicate that a substantial proportion of MDS patients in all international Prognostic Scoring System (IPSS) risk groups are red blood cell transfusion-dependent: Low risk, 47%; Intermediate-I risk, 58%; Intermediate-II risk, 70%; High risk, 82%. Survey responses by European and other non-US centers revealed that an average of 57% of transfusion-dependent patients receive parenteral iron chelation therapy and that the criteria for initiating chelation therapy are not uniform. The number of transfusions was reported as a determining criterion by 47% of respondents, with a mean number of 36 transfusions. 15% of respondents reported that the number of transfusions was their sole criterion for beginning iron chelation therapy. Serum ferritin levels were reported as a determining criterion by 72% of respondents, with the following cut-off values:  

<table>
<thead>
<tr>
<th>Ferritin concentrations for initiating chelation therapy.</th>
<th>% respondents using this cut-off as criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1000 ng/mL</td>
<td>35%*</td>
</tr>
<tr>
<td>&gt;1500 ng/mL</td>
<td>17%</td>
</tr>
<tr>
<td>&gt;2000 ng/mL</td>
<td>35%</td>
</tr>
<tr>
<td>Other (&gt;3000, unspecified)</td>
<td>13%</td>
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</tbody>
</table>

*% respondents using this cut-off as criterion*

32% of respondents indicated that serum ferritin was the sole criterion used. (97% indicated that they monitored ferritin levels in transfusion-dependent patients irrespective of chelation therapy.) Other criteria used to determine start of chelation therapy included age/life expectancy, MDS subtype, clinical signs of hemochromatosis, quantitative CT liver iron estimation, liver function, transferrin saturation >50%, BMT, anticipated chronic transfusion need, and logistical issues and insurance coverage. A combination of criteria was reported to be used by 28% of respondents. **Conclusions.** The decision for initiating chelation therapy in transfusion-dependent anemic MDS patients needs to be individualized because of the heterogeneous patient population. However, this data analysis suggests a need for standardizing select criteria, such as the number of transfusions and serum ferritin, for determining when to initiate iron chelation therapy.

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**Chronic myeloproliferative disorders II**

**0955**
ESSENTIAL THROMBOCYTHEMIA AND PREGNANCY: PRELIMINARY REPORT OF THE PREGNANCY COMMITTEE OF THE REGISTRO ITALIANO TROMBOCITEMIE (RIT)

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**Backgrounds.** Essential Thrombocythemia (ET) is diagnosed in the childbearing age in about 20% of patients. Fertility reduction and adverse outcome of pregnancy due to thrombotic or hemorrhagic complications are a matter of concern. **Aims.** To evaluate the outcome of pregnancy in a large series of patients in order to identify a possible guideline for the management of pregnancy in ET. **Materials and Methods.** The pregnancies observed in ET patients in seven Italian Hematological Centres from 1996 to 2005 were registered in the RIT. Results. Fifty-nine pregnancies occurring in 47 women (age 22-45 years) with ET diagnosed according to the WHO criteria were enrolled. None of the WHO criteria had a recognized thrombophilic abnormality other than ET. Besides 37 live births (66%), 7 first trimester losses (12.5%), 6 second trimester losses (10.7%), 2 still births (3.5%) and 5 voluntary abortions on social grounds were described; 3 pregnancies are ongoing. One case of IUGR and 8 premature births at weeks +26, +28, +32, +34, +36 and +36 were reported. Maternal morbidity in this case series was absent. Thirty-six patients (61%) received Aspirin (100 mg) during the pregnancy and 9 out of them also received prophylactic LMWH for six weeks post-partum. Interferon α treatment was performed in 12 patients with a platelet count >1,000×10^11/L and considered at high thrombotic risk. The outcome of pregnancies in these 12 patients was the following: 12 live births (70.6%), 2 still births, 2 foetal losses (at weeks +8 and +28) and 1 ongoing. Overall there were 6 premature births at weeks +26, +32, +33, +34, +34 and +36 respectively. Pregnancy outcome in the remaining group was the following: 22 live births (55%) 11 foetal losses (27.5%), 5 therapeutic abortions and 2 ongoing. Twenty-three pregnancies occurred among 18 women taking Interferon α (10 cases), Hydroxyurea (5 cases), Anagrelide (7 cases) and Busulphan (1 case). The pregnancy had the same outcome than in the overall population: 16 live births (69.5%), 5 foetal losses (21.7%), 1 premature birth (4.3%), 2 therapeutic abortions and 1 ongoing. **Conclusions.** These data confirm that foetal morbidity and mortality is not negligible in ET. Cytoreductive therapy with Interferon α seems potentially able to protect against foetal losses. Although normal pregnancies have been registered in patients who conceived during cytotoxic treatment, the adoption of effective forms of contraception throughout treatment is still strongly recommended. The epidemiological, clinical and biological data on pregnancy in ET obtained by the participating Centres are now object of prospective study by the RIT (CIMEA project) which records the ET patients diagnosed in Italy since January 2004. Therapeutic options including antithrombotic treatment and cytoreductive therapy will be considered and a management plan for pregnancy in ET will be proposed.

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**0956**
EFFICACY AND SAFETY OF PEGYLATED INTERFERON α IN PATIENTS WITH POLYCYTHEMIA VERA: A PROSPECTIVE MULTICENTER PHASE II STUDY

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**Backgrounds.** Interferon α (IFN) is a therapeutic option for patients with polycythemia vera (PV) to control increased myeloproliferation. For pegylated formulations of IFN neither efficacy nor tolerability have been published in larger series of patients (pts) with PV. Aims. A phase II study has been conducted to investigate the antiproliferative effects and the safety of pegylated IFN α2b (PegIntron) in PV pts. PegIntron α2b was administered subcutaneously with a starting dose of 50 µg/week. Dose escalation every six weeks to 100 and 150 µg/week or
dose reduction was recommended according to response and toxicity. Pretreatment with one cytoreductive drug but not conventional IFN was permitted in addition to phlebotomy. Complete response (CR) was defined as a stable hematocrit ≤45% without phlebotomy, normalization of platelet counts and normal spleen size. Good partial response (PR1) was defined as reduction of phlebotomies and/or platelet counts and/or splenomegaly <50%, poor partial response (PR2) is the respective reduction between 25-49%, Results. Since February 2003, 49 pts (29 m / 20 f) with PV according to the WHO criteria have been enrolled. 21/23 pts investigated were positive for the JAK2(V617F) mutation. Follow-up data are presently available from 37 pts with a median age of 59 (41-78) years and a median duration of therapy of 23 (2-36) months. At their last reported observation, two patients (6%) were heterozygous for the JAK2(V617F) mutation status in 419 patients diagnosed with PV. The aim was to evaluate whether homozygosity is associated with the clinical phenotype and disease progression.

Aims. We used a quantitative real-time polymerase chain reaction (qR-PCR)–based allelic discrimination assay for the evaluation of granulocyte JAK2 (V617F) mutation status in 419 patients diagnosed with a myeloproliferative disorder. Results. JAK2 (V617F) was detected in 135/150 (89%) patients with PV, 62/125 (50%) patients with ET, and 55/91 (60%) patients with CML; in addition, it was found in 31/31 (100%) patients with post-PMF and in 10/25 (40%) patients with post-ET MF. Patients with PV had higher percentages of JAK2 mutant alleles than those with ET, and patients with fibrotic CML had higher values than those with prefibrotic CML; patients with post-PMF myelofibrosis had the highest percentages of mutant alleles. Overall, the longer the time elapsed from diagnosis, the higher the percentage of mutant alleles; sequential studies in a subgroup of patients showed that on average the proportion of mutant alleles increased with time. Granulocyte JAK2 mutant alleles were greater than 50% in all patient samples available prior to therapy and during follow-up. Consequently in a myeloproliferative disorder. By using sensitive assays, the present observations suggest that low proportions of mutant alleles (<25%) are mainly associated with thrombocytosis, intermediate proportions (25-75%) with erythroid hyperplasia, and high proportions (>75%) with myeloid metaplasia and splenomegaly. Physiological and genetic modifiers are expected to further influence the clinical phenotype.

0958

HOMOZYGOSITY FOR JAK2(V617F) IDENTIFIES MPD PATIENTS WITH A MORE SYMPTOMATIC DISEASE. AN ITALIAN GIMEMA RETROSPECTIVE STUDY ON 989 PATIENTS

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Background. An acquired mutation in the JAK2 gene is found at different rates in patients with chronic myeloproliferative disorders (MPD); in about 20% of polycythemia vera (PV) or idiopathic myelofibrosis (IM), and in less than 3% of essential thrombocytopenia (ET), the mutation is harboured in the homozygote status. This low frequency of homozygosity has prevented until now the elucidation of its impact on disease phenotype.

Aims. This large survey of MPD patients, that allowed to evaluate 152 homozygote patients, was aimed at determining whether homozygosity for JAK2(V617F) pointed to a subgroup of MPD patients with unique clinical characteristics. Design and Methods. In an Italian cooperative GIMEMA retrospective study, 989 MPD patients were enrolled from 11 hematology centers. The diagnosis of PV was made in 528 (53%), of ET in 400 (40%), while 254 (26%) were IM and 37 (4%) were post-PV/ET.

Conclusions. Diagnosis of PV or ET was made according to either the PVSG or WHO criteria, while the Consensus Conference Criteria were used for IM. The only eligibility criterion for inclusion was the availability of a JAK2(V617F) mutational status determination according to the ASO-PCR and the BsaXI digestion method (Kratzer 2005). Results. 152 patients (32%) were wild-type (WT), 520 (53%) were JAK2(V617F) heterozygote and 152 (15%) homozygote; among the latter, 81 were PV, 82 ET, 45 IM and 18 PP/PTTTM, accounting for 25%, 2%, 20%, and 49%, respectively, of patients within each diagnostic group. Irrespective of their diagnosis, homozygote patients had a more severe anaemia and hematocrit value, while platelet counts were unchanged. The frequency of splenomegaly progressively increased from 40%, to 51% to 69% in WT, heterozygotes or homozygotes; similarly, the occurrence of purpura rose from 8% to 18% to 28%, and that of systemic symptoms from 25% to 50% to 38%. There were 351 thrombotic events, of which 251 were major events and 187 of the microvessels; major hemorrhages were 45. There was a higher incidence of thrombosis in homozygotes (55%) than in heterozygotes (86%) or WT (26%), while there was no difference in hemorrhages. In PV and ET, homozygosity was associated with a greater risk of evolution into myelofibrosis (12% and 25%, respectively) compared to heterozygosity (2.2% and 2.5%); noteworthy, the highest frequency of homozygosity was recorded among PP/PTTTM patients (49%). Finally, the frequency of patients overexpressing PRV-1 gene was greater among homozygotes (89%) than heterozygotes (69%) or WT (42%). Conclusions. This large survey of MPD patients, that allowed to evaluate 152 homozygote patients, supports the contention that the loss of wild-type JAK2 allele in hematopoietic cells characterizes a quite homogenous category of patients with more symptomatic disease within each MPD diagnostic category. Assessment of JAK2(V617F) homozygosity may have a role in risk prediction and patient management.
E. Lippert, S. Sica, Thrombosis of splanchnic or cerebral veins can develop E. Rossi, V. Praloran, mutation in PV, patients were not Our results Congress of the European Hematology Association mutation was studied in 124 and bone marrow biopsy, many patients were misdiag- N. Boiret, The mutation in 73% and 45% of patients, respectively, whereas it is 19 patients with overt CMD (6 with PV, 12 with ET, and 1 with idiopath- (PMVT), and 39 with cerebral vein thrombosis (CVT). No patient fulfilled vein thr ombosis (HVT), 60 with por tal-mesenteric vein thr ombosis (HVT), 3 patients, PMVT 125 years, range 21-80); clinical manifestation was HVT in 3 patients, PMVT and a thrombocytosis (>400 10 F . Girodon, WHAT IS THE BEST STRATEGY FOR THE DIAGNOSIS OF POLYCYTHEMIA VERA? L.P. Ludmila, G.Y.U. Miterev, K. S. Momotjuk, M.Y. Vakhroucheva, N.V. Tzetvaeva, M.S. Sokolova, A. Turkina, N.D. Khoro National Hematology Research Centre, MOSCOW, Russian Federation Background. Chronic myeloproliferative diseases (CMD) is a group of malignant clonal disorders of haemopoietic stem cells. Malignant haemopoietic cells may exert influence on the stromal cells, which may result in alteration of the haemopoietic microenvironment. Aims. The purpose of the present study was to estimate functional activity of the haemopoietic microenvironment in CMD patients. We have studied the ability of bone marrow stromal cells from CMD patients to support pro- liferation and differentiation of cobblestone areas forming cells (CAFC) of normal individuals. Methods. Stromal and hematopoietic cells were obtained from bone marrow aspirates of 9 CMD patients (4 patients with idiopathic myelofibrosis, IMF and 5 patients with chronic myeloid leukemia, CML) and 6 normal individuals. We used the stromal feeder layers after irradiation (48 Gy): 3-4 week long-term cultures (LTC) and fibroblasts stimulated to osteogenic differentiation by dexamethasone (10^-6 M). The normal haemopoietic cells were seeded on the stromal cells of the CMD patients and of normal individuals. Functional activi- ty of stromal cells of CMD patients was estimated by the number of cob- blestone areas-forming cells (CAFC) on different stromal feeder layers in LTC bone marrow by limiting dilution assay. Stromal cells of the normal individuals were used as a control. The late (1-5 weeks incubation) and early (6-9 weeks incubation) CAFCs were estimated. Results. Our results showed that all examined stromal cells from IMF and CML patients sup- ported growth of the CAFCs of the normal individuals up to 9 weeks incubation. We have detected that number of the early (0,6±0,3) and late (4,8±0,7) CAFCs was decreased on stromal layers of LTC of the IMF patients in comparison with the normal stromal layers of LTC (early - 7,5±1,1 and late -45,3±6,1) CAFCs. The number of the early CAFCs (4,4±1,7) was reduced on IMF patient fibroblasts stimulated to osteogenic differentiation too, whereas the number of the early CAFCs on the stimulated normal fibroblasts was 10,2±1,9. The number of the early and late CAFCs in the CML patients was as on the normal stromal cells. Conclusion. IMF patient stromal cells can not support growth of the normal CAFCs, suggesting that the haemopoietic microenviron- ment have a functional defect in IMF patients.
0962  GENE EXPRESSION PROFILING IN ESSENTIAL THROMBOCYTHEMIA USING CDNA MICROARRAY TECHNOLOGY. PRELIMINARY RESULTS
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Background. Essential thrombocytopenia (ET) is a chronic myeloproliferative disorder (CMPD) lacking specific molecular markers. Consequently, its diagnosis is based on exclusion of other CMPD and secondary thrombocytosis. Aim. The aim of the study was to characterize the gene expression profiling in ET using cDNA microarray technology, specifically analyzing the implication of the JAK-STAT signaling pathway.

Patients and Methods. Peripheral blood granulocytes obtained from 20 ET patients diagnosed according to the PVSG criteria (1997), who had not received platelet-lowering therapy, were isolated. Good quality RNA (RIN>7) was hybridized competitively to the RNA granulocytes obtained from ET patients (ET0) and from healthy donors (H0) and from 15 previously published samples. Duplicate morphologies were performed with dye-swap to control for possible differences in the incorporation rate of the two fluorochromes. Oligonucleotide cDNA microarray expression profilings were obtained using 44K whole human genome oligo microarrays (Agilent Technologies, Palo Alto, CA), comprising 41,000 oligonucleotides. Fluorescent images were obtained using an Agilent G2565BA scanner and Genepix 6.0 (Axon Inc.) was used to extract data from the image and analysis was performed using the R-package. Results. From 41,000 oligonucleotides covered in the Agilent platform, an homogenous TE signature was obtained in 17 out of 20 patients studied. This signature was composed of 124 genes that were found to be more than 2-fold up-regulated and 14 genes 2-fold down-regulated, in relation to control granulocytes. The other 8 patients showed a distinct expression profile and different to each other. Of the 124 up-regulated genes, 101 had an assigned function, being the immune response the most implicated, with 29 genes overexpressed. In addition, cellular movement (28 genes) and hematological system development and function (28 genes) were also involved. The most important involved network comprised 35 genes, mainly CXCL2, CD83, PTGS2, CCL3L1, GCH1 and TNFAIP3, which mediate immune and inflammatory responses. Two other networks also implicated cellular growth and proliferation, cellular movement and hematological system development and function (13 genes, being the most important DUSP3, MAP2K5, DUSP2), cellular protein metabolism (TBCG, SOLH), cellular localization and intracellular transport (AF3M1), PRV-1, c-MPL and TPO gene expression was not affected in any of the 20 patients studied. Interestingly, only one gene (CXCL2) involved in the JAK-STAT signaling pathway was affected. No differences regarding expression patterns were found between ET patients with and without the JAK2 V617F mutation. Conclusions. Our preliminary results have shown an homogeneous expression pattern in 17/20 ET patients. It is remarkable that an important number of genes were up-regulated, most of them being implicated in the immune response, cellular movement and hematological system development and function.

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0964  MOLECULAR ANALYSES IN FAMILIAL AND SPORADIC CONGENITAL PRIMARY ERYTHROCYTOSIS
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Backgrounds. The only molecularly characterised type of primary familial and congenital erythrocytosis/polycthemia (PFCP) is caused by dominant mutations in the erythropoietin-receptor gene (EPOR). EPOR mutations are estimated to account for 12-15% of cases with congenital primary erythrocytosis. So far, at least fourteen different EPOR mutations have been described, eleven of them leading to a truncation of the intracellular part of the receptor, resulting in hypersensitivity of erythrocyte progenitors to circulating Epo. The majority of previously reported mutations has not been identified in additional patients outside of the original family. Aim. To search for the underlying genetic base in patients with familial or sporadic congenital primary erythrocytosis of so far unexplored origin: 1. Analysis of EPOR to identify unknown mutations or any of the previously reported mutations if occurring independently of the original family. 2. In patients without EPOR changes, exclusion of a somatic or genetic variant of the JAK2 V617F mutation which was previously detected in patients with polycythemia vera (PV) and other myeloproliferative disorders. Method: 16 patients (age range 5-66 years) with a serum Epo level of < 10 mU/mL have been included in this study, 3 of them being related (a mother and two of her sons). P. vera was excluded according to FVSG or WHO diagnostic criteria. Sequencing analysis of coding regions and intron/exon boundaries of the EPOR gene was performed on genomic DNA. An allele-specific PCR was used to exclude or diagnose the JAK2 V617F mutation. Results. An EPOR mutation 1453G→A creating a termination signal at codon 459 (Trp459Ter) was
found in a 5 year old Spanish girl. Her parents and her brother do not present this mutation and have normal blood counts. Another EPOR mutation (EPOR 1414C→G, Tyr426ter) was detected in the multimember family case. Interestingly, the mother currently presents with normal hemoglobin and hematocrit levels (only mild microcytosis). She had been included in the study because of her affected sons but also because of her history of polycythemia during childhood and adolescence. This change of the clinical presentation is of particular interest since the original identification of this mutation in first PPCP family had been complicated by the fact that one family member with the mutation was apparently clinically unaffected. None of the patients presented the JAK2 V617F mutation. Conclusion: EPOR gene mutations causing either sporadic or familial primary congenital erythrocytosis are found in patients of various ethnic origins. Considering the fact that 3 of the 16 patients were from one family, the previously reported prevalence of EPOR gene mutations in the group of primary erythrocytosis of about 15% is confirmed by this study. The mutation EPOR 1414C→G previously described was independently detected in a second family and is associated with a variable phenotype. The exploration of the underlying physiological mechanisms may contribute essentially to the knowledge about erythropoiesis’ regulation. The PV-characteristic mutation JAK2 V617F does not seem to play a role in congenital primary erythrocytoses.

**0965**

**THE LEVELS OF JAK2V617F RNA DICTATE THE CLINICAL PHENOTYPE IN POLYCYTHEMIA VERA AND IDENTIFIES PATIENTS WITH MORE SYMPTOMATIC DISEASE**


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**Background.** The occurrence of an unique JAK2 V617F mutation in phenotype-distinguishable chronic myeloproliferative disorders (MPD), including polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (IM), suggests that other genetic events/gene modifiers might be involved. **Aims.** As an approach to unravel significant associations between phenotype and the JAK2 mutation, we have correlated the levels of JAK2 V617F RNA with clinical and laboratory characteristics at the diagnosis in 63 patients with polycythemia vera (PV) and 115 with essential thrombocythemia (ET), as diagnosed according to the WHO criteria. **Methods.** Wild-type and mutated JAK2 RNA levels were determined by an amplification-refractory mutation sequencing (ARMS) PCR assay on granulocytes, and expressed as the percentage of mutated JAK2 RNA over total (Vannuchi AM et al, Leukaemia, In press). **Results.** 53/65 PV patients (84%) and 76/115 (66%) presented detectable levels of JAK2 V617F RNA; the amount of mutated RNA was higher in PV than in ET granulocytes (median 52% and 12.5%, respectively; p<0.0001). In PV patients, the hematocrit and white blood cell count were significantly related to the amount of mutated RNA, while there was an inverse relationship with MCV and platelet counts. None of these parameters were significantly correlated with mutated RNA levels in ET. **Conclusion.** When the analyses were restricted to those PV patients who showed RNA levels in a range similar to that observed in ET (1-55%) the above correlations were maintained, thus ruling out that these effects might be simply ascribable to the overall higher load of JAK2 V617F RNA in PV than in ET patients. Among PV patients with JAK2V617F mutation, the frequency of splenomegaly, of therapy (Rebotomies and chemotherapy) and of chemotherapy requirement were all significantly increased over wild-type patients, but again not in ET pts. On the other hand, in both PV and ET JAK2 V617F mutated patients there was a great frequency of EEC and overexpressed PRV1 gene, while there was no difference in CD34+ cell count in the peripheral blood. The percentage of high-risk patients was higher among mutated than wild-type ones (63% vs 27%, p=0.003) if patients were all considered together, but did not reach the significance level in the ET group alone (62% vs 38%, p=0.07). In the contrary, in PV there was a progressive increase in the percentage of high-risk patients according to the amount of mutated RNA (10% in wild-type, 24% in patients with 1-25% JAK2 V617F RNA and 66% among those showing 26-100% JAK2 V617F RNA). **Conclusions.** By quantifying the amount of JAK2 V617F RNA in granulocytes, we documented a gene dosage-effect in PV, but not in ET, suggesting that the JAK2 V617F mutation appears in the polycythemic phenotype in PV patients while additional genetic or host factors modulate the disease presentation in ET. Also of note, the levels of JAK2 V617F RNA identified PV patients with more symptomatic disease in terms of blood abnormalities, therapy requirement and high-risk category.

**0966**

**DIAGNOSIS OF ESSENTIAL THROMBOCYTHESIA: THE USEFULNESS OF JAK2 V617F MUTATION DETECTION IN PLATELETS RNA**

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**Backgrounds.** The discovery of the JAK2 V617F mutation has profoundly modified the diagnosis of myeloproliferative diseases (MPD). In essential thrombocythemia (ET), the most frequent MPD, the mutation has been found in 30 to 67% of cases studied neutrophil’s DNA. Aim: In this study we aimed to assess the most informative cellular fraction for the JAK2 V617F detection. Patients and Methods. We explored a cohort of 260 consecutive patients referred to our institution with a suspected diagnosis of ET. We studied neutrophils and bone marrow mononuclear cells at the DNA level and platelet’s RNA. The detection of the mutation consisted in a real time PCR on LightCycler followed by a melting curve analysis (sensitivity 2-4%), allowing a semi-quantitative estimation of mutated/wild type allele. Bone marrow culture assays for endogenous erythroid and megakaryocytic colony formation (ECC, EMC) were performed in 146/260 patients. Results. The mutation was found in 141/260 (90%) patients. In 82 patients both neutrophils and platelets were studied. In 52/82 patients the mutation was detected in neutrophils and 40/82 in platelets (p=0.18). Thus 8 patients were detected only in platelet’s RNA. Using an optimised assay (sensitivity 0.8%) all of them were found mutated in neutrophils. However these patients were more easily detected in platelets. Furthermore, 16/32 (50%) had no more than 10% JAK2 V617F allele in neutrophils. In comparison only 2/27 PV patients had the same profile. In 27 patients studied both in platelets and bone marrow, no difference was found for JAK2 mutational status. Bone marrow cultures revealed ECC or EMC in 6/74 (86%) of JAK2 mutated samples. ECC or EMC were also found in 15/46 (33%) JAK2 V617F negative samples in platelets or bone marrow. Conclusion. In the context of suspect ET, JAK2 V617F mutation is more easily detected in platelets. The presence of ECC or EMC in JAK2 non mutated cases supports the idea of another underlying molecular defect. Therefore bone marrow cultures and morphology remain usefull for the diagnosis of these MPDs.

**0967**

**UPDATE OF THE GERMAN ESSENTIAL THROMBOCYTHEMIA (ET) STUDY: INCIDENCE OF COMPLICATIONS DURING LONG-TERM FOLLOW-UP**

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The German ET-Study is a prospective, randomized multicenter trial with 31 participating centers recruiting 123 patients until 1999. Patients were stratified according to a previous history of ET related complications or a platelet count > 1500 G/L in high or low-risk ET patients. ET patients were regarded as high-risk ET patients, if there has been a previous history of ET related complications or if the platelet count was > 1500 G/L. These patients were randomized either to interferon α (IFN) or hydroxyurea (HU). Low risk ET-patients were defined as ET patients with no ET related symptomatology or a platelet count < 1500 G/L. These patients were observed until ET related complications did occur or until the platelet count increased above 1500 G/L. In total 123 patients with a newly diagnosed ET according to the PVSG criteria and no prior cytoreductive treatment were recruited. Out of these 123 patients, 55 had a high-risk ET and were randomized to either HU (n=27) or IFN (n=28). The remaining 68 patients had a low-risk ET. After a median follow-up of 6 years (range 1-10 years) 13 low risk ET patients developed ET-related complications (6 thromboembolic episodes and 5 microcirculatory disturbances). This resulted in a total complication rate of 3.5% per 100 patient-years and in a rate of 2.0% per 100 patient-years for thromboembolic complications alone. No major bleedings were observed. ET-related complications were significantly dependent on age (age > 60 years, p=0.001) or the presence of ≥2 cardiovascular risk factors (p=0.006). After a median follow-up of 6.6 years (range 0.2-11.1 years) the total complication rate in high risk patients was 5.2% per 100 patient-years for IFN treated patients and 6.5% per 100 patient-years for HU treated patients (p=0.5). Age > 60 years (p=0.01) or the presence of ≥1 cardiovascular risk factors (p=0.026) were associated with a significant higher risk of ET related complications in all high risk patients. After 3 years, half of the
IFN treated patients discontinued IFN due to side effects. Two patients transformed into blast crisis while on HU. There was no patient in the IFN group developing a blast crisis. In summary, HU remains the standard treatment for high risk ET patients although the potential leukemogenicity of this drug still remains a matter of concern. In low risk ET patients a watch and wait strategy is still justified. Higher age (> 60 years) and/or the presence of cardiovascular risk factors at diagnosis are significantly associated with a higher complication rate in both high and low risk ET.

0968

A PHASE II STUDY OF Nilotinib (AMN107), A NOVEL TYROSINE KINASE INHIBITOR, ADMINISTERED TO PATIENTS WITH SYSTEMIC MASTOCYTOSIS

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Backgrounds. Systemic mastocytosis is a clonal disorder characterized by constitutive activation of c-Kit based on point mutations and is characterized by mast cell infiltration of extracutaneous organs. Nilotinib is a novel aminopyrimidine which potently inhibits Bcr-Abl, as well as the PDGF-R and c-Kit kinases.

This study was designed to evaluate the safety and efficacy of nilotinib administered at an oral dose of 400 mg twice daily. Methods. This is a Phase II, open-label study of SM patients with specific disease criteria and with a clinical indication for treatment. Results. Preliminary data are available for the first 23 (11 f, 12 m) out of 55 patients currently enrolled in the study. The median age is 49 (range 33-73) years and the median time from diagnosis of SM was 27 (range 1 to 292) months. Of the patients with data available 17 pts had a c-kit D816V mutation in bone marrow cells or extracutaneous organs. The median exposure to nilotinib was 144 days. Treatment is ongoing for 18 (75%) patients, 5 (22%) have discontinued, 3 (13%) for adverse events and 2 (9%) withdrew consent. There were three (13%) responses reported (2 complete remission and 1 minor response) based on serum tryptase, bone marrow mast cell counts and improvement of clinical symptoms. Baseline mutation data are available for 2 of the 3 responding patients and revealed the c-kit D816V mutation. Anemia was reported in 2 (9%) patients. Adverse events occurring in ≥10% of patients included headache 52% (n=12), fatigue 39% (n=9), nausea 35% (n=8), vomiting, pruritus 30% (n=7 each), muscle spasms 26% (n=6), diarrhea, upper abdominal pain, rash 22% (n=5 each), dizziness, extremity pain 21% (n=5 each), dyspnea, myalgia, increased ALT 17% (n=5 each), bone pain, abdominal pain, cough, hard feces, pustular rash 3% (n=3 each), hypoten- sion, fever 2% (n=1 each) and hypertension 1% (n=1 each). Overall grade 3/4 adverse events included headache, pruritus, hypotension, dyspnea, myalgia, increased ALT 9% (n=2 each), fatigue, muscle spasms, diarrhea, dizziness and extremity pain 4% (n=1 each). There were no deaths. Summary/Conclusions. These data suggest that nilotinib has clinical activity and an acceptable safety and tolerability profile in patients with systemic mastocytosis.

0969

IMATINIB-MESYLATE THERAPY FOR SYSTEMIC MASTOCYTOSIS: RELATIONSHIP TO C-KIT MUTATIONAL STATUS

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Background. Systemic mastocytosis (SM) includes an heterogeneous group of neoplastic disorders characterized by an abnormal mast cell accumulation in various tissues and by both indolent or aggressive clinical outcome. SM has been supposed to be associated with two classes of constitutive activating c-kit somatic mutations: the so-called enzymatic site type (EST) mutations, affecting the structure of the catalytic portion of the kinase (e.g. D816V), and the regulatory type (RT) mutations affecting the regulation of an otherwise normal catalytic site (e.g., V560G). Aims. Since c-kit is a transmembrane receptor-type tyrosin kinase, we aimed to test the hypothesis of whether an inhibitor block-
detected in 14, only 1 of whom had a secondary MMM (p=0.02); such patients showed higher values of leucocyte count (p=0.18), CD34 count (p=0.03), serum LDH (p=0.02) and spleen size, a higher frequency of severe anaemia (hemoglobin <9g/dL; p=0.02), higher SDF1 plasma levels (p=0.04), lower percentage of CD34 + CXCR4 + cells, higher percentage of CD34 + intra/CXCR4 cells and a trend to a lower bone marrow cellularity, as compared with SDF1-AG or AA patients. Similar differences between the GG and the AG/AA genotypes were detected both in idiopathic and secondary MMM patients, and also in the patients with mutated JAK2. Conclusions. The SDF1-3'A polymorphism is highly frequent in secondary MMM and influences MMM phenotype, favoring a less intense myeloproliferation and less severe anemia.

0971. TOPOGRAPHY OF INTRAMEDULLARY HEMATOPOIESIS IN MYELOFIBROSIS WITH MYELOID METAPLASIA: RELEVANCE OF 99TC-BW250/183 IMMUNOSCINTIGRAPHY

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Backgrounds. Myelofibrosis with myeloid metaplasia (MMM) is a rare chronic myeloproliferative disease characterized by both myeloproliferative and myelodystrophic features: myeloproliferation typically includes enhanced spontaneous mobilization of hematopoietic progenitor cells (HPC) from the bone marrow (BM) and their homing into extramedullary sites (mainly spleen); myelodysplasia results from exhaustion of both BM and extramedullary hemopoiesis. Aims. To investigate the extent and distribution of hemopoiesis in MMM patients and to capture its relationship with BM fibrosis, HPC mobilization and clinical severity. Methods. Immunoscintigraphy employing a dual-head camera was performed 120-260 minutes (median 180 minutes) after administration of 553-830 MBq (median 700 MBg) 99mTc-BW250/183, corresponding to 0.3-0.5 mg. Hemopoietic function in the central compartment: they had a higher WHO fibrosis grade (p=0.008), spleen size (9.3 vs 2.4 centimeters from costal arc; p<0.1%; no blasts; ≤0.1% immature myeloid cells). From these data it was concluded that the patients with mutated JAK2. Conclusions. The SDF1-3'A polymorphism is highly frequent in secondary MMM and influences MMM phenotype, favoring a less intense myeloproliferation and less severe anemia.

0972. THE REGISTRO ITALIANO TROMBOCITEMIA: PRELIMINARY ANALYSIS OF THE FIRST 650 ENROLLED PATIENTS

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Background. Many epidemiological, diagnostic, prognostic and therapeutical data were obtained by the Italian Registry with the retrospective study performed in over 2000 Essential Thrombocythemia (ET) patients, mainly diagnosed according to the PVSG criteria and treated with potentially leukemogenic drugs. Now are claimed updated data in ET patients diagnosed according to the WHO criteria and treated more largely with non leukemogenic molecules. Aims. The RIT belonging to the GiMEMA Group has been activated in order to register Italian ET patients to improve the diagnosis appropriateness (WHO criteria) by performing a centralized revision of the bone marrow biopsies; to promote the acquisition of biological data; to evaluate the compliance to the therapeutic guidelines of SIE, SIES, GITMO; to monitor particularly the ET patients receiving Interferons α and Anagrelide; to evaluate cases of pregnancy, pediatric age and familiarity; to identify new prognostic factors (JAK2 mutation, clonality, etc); to create a network for activation of new clinical and biological studies. Methods. The RIT, co-ordinated by the Hematology Unit of Reggio Emilia, is a web-based registry that besides a public area comprehends a database of Italian ET patients. The data, with respect of the privacy rules, are object of validation and analysis by various RIT Expert Subcommittees. Results. Eighty Hematological Centers adhered to the RIT and 650 patients have been registered since June 2005. In the first 505 analysed cases the ET diagnosis was done according to the PVSG (92%) and WHO (8%) criteria. The patients, 311 females and 194 males, had age <40 yr (17%), 40-60 yr (34%), 60-70 yr (20%), >70 yr (29%) with median age 59 years. At diagnosis the platelet count was >1000x10^9/L in 27% of cases (mean 915). Few patients had prior thrombosis (4.4%; major 2.4%) and prior hemorrhage (1%). The rate of high risk patients, on considering age >60 yr and/or previous thrombosis and/or PLT count >1500x10^9/L, was 56% (64% on considering the PLT count cut-off of 1000x10^9/L). The patients shown general thrombotic risk factors (69%), disease related symptoms (42%) and splenomegaly (27%). Data on the bone marrow biopsy permitted to identify as true ET (WHO criteria) the 27% of the cases. The cytogenetic study documented a normal karyotype in all the 274 evaluated cases. The bcr-abl transcript was absent in all cases. Fifty-nine pregnancies have been reported. Aspirin was administered in 70% of cases and cytoreduction was performed in 63% of cases: Hydroxyurea 61%, Anagrelide 12%, Interferons α 11%, Pipobroman 4%, Busulfan 2%. The follow-up is too short to analyse data. Conclusions. Many (80) of the Italian Hematological Centers have been accredited by the RIT. In 92% of ET patients diagnosis was still done according to the PVSG criteria, but the ongoing revision of the bone marrow biopsies will permit a reclassification according to the WHO criteria. Improvement of the diagnostic approach is expected since the harvest of biological material has been activated. A separate analysis is ongoing for specific series of patients treated with anagrelide and interferons α.
Familial Chronic Myeloproliferative Disorders: Clinical Phenotype and JAK2 (V617F) Mutation Status

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Background. Philadelphia (Ph)-negative chronic myeloproliferative disorders (CMD) include polycythemia vera (PV), essential thrombocythaemia (ET) and chronic idiopathic myelofibrosis (CIMF). CMD are acquired diseases due to a somatic stem cell mutation leading to clonal expansion of myeloid precursors. A gain-of-function mutation of the Janus kinase 2 (JAK2) gene has been recently recognized as a pathogenetic event of CMD. Besides sporadic cases, one or several CMD may affect different relatives of the same family, namely familial CMD. The probability that two CMD occur in the same family as independent events is really low (estimated annual incidence: $10^{-5}$). This suggests the presence of genetic predisposition for somatic mutations leading to CMD-like syndromes in families. Although familial cases carrying JAK2 (V617F) mutation have been reported, the frequency of this mutation in CMD families and its role in disease-causing remain to be defined.

Aims. The aim of this study was to evaluate the clinical features and outcome of familial chronic myeloproliferative disorders, to assess the frequency of JAK2 (V617F) within familial cases, and to define its role in disease-causing. Patients and Methods. Sixteen pedigrees were evaluated for clinical and molecular studies. Pedigrees included 11 families with an homogeneous phenotype (polycythemia vera in 8; essential thrombocythaemia in 3), and 5 with a mixed CMD phenotype. Collecting DNA from granulocytes and T lymphocytes, we detected JAK2 (V617F) by use of quantitative mutation-specific polymerase chain reaction with X-chromosomal clonality markers HUMARA, PGK, and IDS.

Results. Clinical features at diagnosis and outcome did not differ between familial and sporadic CMD. JAK2 (V617F) ranged from 5.3 to 91.5%: the higher value being detected in post-polycythemia myelofibrosis. Distribution of mutant JAK2 within the same pedigree displayed an homogeneous pattern (5 families), or a discordant one (4 families). T cells DNA did not carry mutant alleles. All clonal CMD females, except one with ET, were JAK2 (V617F)-positive. One polyclonal ET was JAK2 (V617F)-positive (low gene dosage: 5.3%). One PV patient was polyclonal and JAK2 (V617F)-negative. Screening of healthy relatives identified 2 subjects with early-polycthemia. Conclusions. These data show that patients with familial CMD have clinical features and outcome overlapping with those of sporadic cases. An extended study of the pedigrees of CMD patients is warranted to ascertain the real frequency of familial cases. JAK 2 (V617F) is a somatic mutation that at least in a portion of familial patients with ET or CIMF does not appear the disease-initiating event.
0975 CYTOGENETICS AND AGE ARE THE MAIN DETERMINANTS OF OUTCOME IN INTENSIVELY TREATED ACUTE MYELOID LEUKEMIA PATIENTS OLDER THAN 60 YEARS: RESULTS FROM AMLSG TRIAL HD98-B
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Backgrounds. Karyotype at diagnosis provides the most important prognostic information in younger adults with acute myeloid leukemia (AML). However, there are few data available looking in particular at patients (pts) above 60 years of age. Aims. Evaluation of the prognostic value of cytogenetics and additional variables in elderly AML patients. Methods. We prospectively analyzed 581 elderly pts with newly diagnosed AML. Chromosome banding was performed using standard techniques. To improve cytogenetic diagnostics, all specimens were also analyzed by FISH using a comprehensive DNA probe set for the detection of the most relevant AML-associated genomic aberrations: inv(3)(q21;q26), t(8;21), t(9;22), t(9;11)(q22), t(11;17), inv(16)(16;16), +3q, -4q, del(5q), del(7q), +8q, +11q, abn(12p), del(13q)/+13q, del(17p), del(20q), +21q, +22q. Results. All pts were treated within the AMLHD98B trial and received intensive induction and consolidation therapy. Pts exhibiting a t(15;17) received an age-adjusted AIDA-regimen. Median follow-up time was 57 months. The median age was 67 years (range 60-85 years). Results. 161 pts. had a normal karyotype (45%); 48 pts. (13%) exhibited the balanced translocations t(8;21)(n=12), inv(16)(n=14), t(15;17)(n=11), or t(11q23)(n=11); in the absence of these balanced translocations, 78 pts. exhibited a single aberration, 179 pts. two aberrations, and 61 pts. a complex karyotype (25 aberrations, including 44 pts. with 5 or more mutations). Analyses were performed on day 8-21 (arm 1) or day 1-7, 15-21 (arm 2), plus DA and HD-ARA. OS [t(15;17) excluded] revealed two risk groups: standard-risk [normal karyotype, t(8;21), inv(16), and t(11q23)] did not differ from pts. with normal karyotype. Pts. exhibiting a t(15;17) showed a significantly better OS (77%) compared to 55% in pts. with other balanced translocations (log-rank test, p<0.001). Stratification of the patients according to cytogenetic risk group and age as dichotomized variable resulted in 5 prognostic groups: i) APL CR 75%, OS 55%, ii) <70 yrs./standard risk CR 62%, OS 24%, iii) <70 yrs./high risk CR 21%, OS 6%, iv) >70 yrs./standard risk CR 89%, OS 5%, v) >70 yrs./high risk CR 15%, OS 2%. Conclusions. Our risk classification system based on cytogenetics and age identified a large proportion of elderly patients with AML who did not benefit from intensive chemotherapy.

0976 5-AZACITIDINE INDUCES REMISSEMS IN PATIENTS WITH TRANSFUSION DEPENDENT MYELOPROLIFERATIVE DISEASES AND IN PATIENTS WITH ACUTE MYELOID LEUKEMIA REFRATORY TO OR NOT ELIGIBLE FOR INTENSIVE CHEMOTHERAPY
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Backgrounds. Epigenetic modulation of gene function is a powerful cellular mechanism. An association between methylation of the p15 ink4b gene promoter and risk for acute myeloid leukemia (AML) transformation in myelodysplastic syndrome (MDS) has been suggested. The DNA hypomethylating agent azacitidine is an active agent in the treatment of MDS and leukemia. AML patients with normal karyotype and recurring epigenetic aberrations are at high risk for developing AML. The treatment efficacy of azacitidine in this cohort is not fully elucidated. Methods. To improve cytogenetic diagnostics, all specimens were also analyzed by FISH using a comprehensive DNA probe set for the detection of the most relevant AML-associated genomic aberrations: inv(3)(q21;q26), t(8;21), t(9;22), t(9;11)(q22), t(11;17), inv(16)(16;16), +3q, -4q, del(5q), del(7q), +8q, +11q, abn(12p), del(13q)/+13q, del(17p), del(20q), +21q, +22q. Results. All pts. were treated within the AMLHD98B trial and received intensive induction and consolidation therapy. Pts exhibiting a t(15;17) received an age-adjusted AIDA-regimen. Median follow-up time was 57 months. The median age was 67 years (range 60-85 years). Results. 161 pts. had a normal karyotype (45%); 48 pts. (13%) exhibited the balanced translocations t(8;21)(n=12), inv(16)(n=14), t(15;17)(n=11), or t(11q23)(n=11); in the absence of these balanced translocations, 78 pts. exhibited a single aberration, 179 pts. two aberrations, and 61 pts. a complex karyotype (25 aberrations, including 44 pts. with 5 or more mutations). Analyses were performed on day 8-21 (arm 1) or day 1-7, 15-21 (arm 2), plus DA and HD-ARA. OS [t(15;17) excluded] revealed two risk groups: standard-risk [normal karyotype, t(8;21), inv(16), and t(11q23)] did not differ from pts. with normal karyotype. Pts. exhibiting a t(15;17) showed a significantly better OS (77%) compared to 55% in pts. with other balanced translocations (log-rank test, p<0.001). Stratification of the patients according to cytogenetic risk group and age as dichotomized variable resulted in 5 prognostic groups: i) APL CR 75%, OS 55%, ii) <70 yrs./standard risk CR 62%, OS 24%, iii) <70 yrs./high risk CR 21%, OS 6%, iv) >70 yrs./standard risk CR 89%, OS 5%, v) >70 yrs./high risk CR 15%, OS 2%. Conclusions. Our risk classification system based on cytogenetics and age identified a large proportion of elderly patients with AML who did not benefit from intensive chemotherapy.

0977 PHASE IB STUDY OF PKC412, AN ORAL FLT3 KINASE INHIBITOR, IN SEQUENTIAL AND SIMULTANEOUS COMBINATIONS WITH DAUNORUBICIN AND CYTARABINE INDUCTION AND HIGH-DOSE CYTARABINE CONSOLIDATION IN NEWLY DIAGNOSED PATIENTS WTH AML
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Backgrounds. Activating mutations in FLT3 (fms-like tyrosine kinase), either an internal tandem duplication (ITD) in the juxtamembrane region or a point mutation in the activation loop, occur in leukemic blasts from 25-35% of AML patients, are associated with poor prognosis, and represent an attractive therapeutic target. PKC412 is a potent, orally active, FLT3 kinase inhibitor which has clinical activity in mutant (reduction in peripheral blasts in 70%) and wild type (reduction in peripheral blast in 80%) AML, but rarely produces remissions (Stone et al., Blood 2005). Aims and Methods. We combined DA induction (daunorubicin 60 mg/m2 d 1-3 and cytarabine 100 mg/m2/d by IVCI x 7d) and post-remission HD-ARA (cytarabine 3 g/m2/3h q 12h, d1,5,5 for 3 cycles) plus PKC412 in newly diagnosed FTL3 mutated (FLT3mut) and FTL3 wild type (FLT3WT) AML patients < 60 years old in a Phase Ib trial to investigate toxicity and efficacy. Results of earlier experience using PKC412 100 mg po bid were reported previously (Giles et al., ASH 2004). This is an updated report of 29 patients treated with PKC412 at a reduced dose of 50 mg po bid given on day 8-21 (arm 1) or day 1-7, 15-21 (arm 2), plus DA and HD-ARA. Eight out of 23 patients (35%) were FLT3mut, and 15/23 (65%) were FLT3WT as determined by D-HPLC. Results. None of the 19 patients evaluated for safety had drug-related death; the most common toxicities were transient elevations of glucose (16%), AST (16%), bilirubin (11%), ALT (11%), and decreases in potassium (21%), phosphate (11%) and calcium (11%); no grade 3 or 4 nausea, vomiting or pleural effusion were recorded. Twenty-nine patients were evaluable for response: 9/12 (75%) achieved CR in Arm 1 and 9/11 (82%) achieved CR in Arm 2. Seven out of 13 (54%) achieved CR and 11/15 (73%) FLT3WT and 7/8 (88%) FLT3mut patients achieved CR. In patients with FLT3WT, remissions were achieved in 17 patients. In patients with AML, remissions were achieved in 16 patients (46%) (CR, n=4, PR, n=2). Stable disease was achieved in 2 patients. Five (38%) patients were refractory to treatment. For patients with MPD, PR was achieved in 3 patients and stable disease in one. For the entire cohort, 55% of transfusion dependent anemias and thrombocytopenias resolved under therapy. Interestingly, complete remissions in patients with AML were achieved after 1-2 treatment cycles. Patients refractory to conventional chemotherapy tended to do worse than those who received azacitidine as first line treatment. Conclusion: Azacitidine applied in an outpatient setting to patients with AML and MPD with a dismal prognosis was well tolerated and could induce complete and partial remissions. It may be a promising new treatment modality for patients with AML and MPD. A larger number of patients and longer follow up are needed to confirm these data, define the number of treatment cycles required and clarify whether a leukemia-free survival correlates with an improved overall survival in this group of patients.
ARSENIC TRIOXIDE (ATO) IS SAFE AND EFFECTIVE IN COMBINATION WITH LOW-DOSE ARA-C (LDAC) FOR THE TREATMENT OF ADVANCED MYELODYSPLASTIC SYNDROME (MDS) AND POOR-PROGNOSIS ACUTE MYELOID LEUKEMIA (AML) IN ELDERLY PATIENTS

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Background/Aims. Treatment outcomes for advanced MDS and elderly AML patients are generally poor. LDAC in elderly AML patients results in a CR rate of approximately 20% and possibly improved morbidity and mortality compared to conventional chemotherapy or supportive care. In MDS patients, the CR rates with LDAC are lower (10-20%) with short duration and no clear benefit over supportive care. On the basis of preclinical data suggesting a possible anti-angiogenic effect of ATO, as well as clinical data showing activity of ATO in MDS, a phase I/II study of ATO in combination with LDAC was initiated in IPSS Int-2/high risk MDS and newly-diagnosed, poor-prognosis AML patients.

Methods. ATO was given at a dose of 0.25 mg/kg for days 1-5 and 8-12. LDAC was dose-escalated from 8 mg/m2 SC BID to the target phase II dose of 10 mg/m2 SC BID for days 1-14 (one treatment cycle). Patients who achieved CR after one treatment cycle were given a second, identical cycle, followed by maintenance treatment of 5 days of LDAC. Patients who did not achieve CR after one cycle were given a second cycle beginning between days 21-28, with the addition of ascorbic acid 1g IV within 30 minutes of the ATO infusion. Results. Eighty-three patients have been enrolled to date, 52 with AML and 31 with MDS. A total of 75 patients (49 AML, 26 MDS) are evaluable for response, 69 (46 AML, 23 MDS) of whom were treated with the target dose of LDAC. There were no responses in the 6 patients treated with less than the target dose. Clinical characteristics of the 46 evaluable AML patients treated at the target dose include: mean age 73 yrs (range 55-85 yrs; one patient < 60 yrs with AML and multiple medical comorbidities was included); abnormal cytogenetics 29 (66%); antecedent hematologic disorder 29 (68%); secondary disease 7 (15%); CR was achieved in 17 patients (37%) and CRp in 1 patient, for an overall response rate of 39%, with follow-up 1-11+ mos. Eight patients (44%) required 2 treatment cycles to achieve CR/CRp. Of the 46 AML patients, 5 died prior to day 30 (induction mortality = 11%), 3 (7%) of progressive disease and 2 (4%) of neutropenic sepsis. Clinical characteristics of the 25 evaluable MDS patients include: mean age 70 yrs (range 56-84 yrs); abnormal cytogenetics 17 (81%); prior therapy with 5-azacytidine 3 (13%). CR was achieved in 6 (26%) patients, follow-up 3-9+ mos. Three patients (13%) required 2 treatment cycles to achieve CR; there was 1 induction death (4%). Summary. The regimen was generally well-tolerated, with minimal grade 3/4 non-hematologic toxicity and no significant nausea, emesis, diarrhea or mucositis. Alopecia was not seen. Grade 4 hematologic toxicity was observed in all patients. Fluid retention occurred in 56/69 (81%) of patients. There were no clinically significant drug-related arrhythmias. The CR rate in AML was comparable to conventional chemotherapy, with improved tolerability and induction mortality, further investigation is warranted.
Acute lymphoblastic leukemia

**0980**

**SINGLE NUCLEOTIDE POLYMORPHISMS OF THE MTHFR (C677T), MTRR (A66G) AND VITAMIN D RECEPTOR (CD2-2/GATA) GENES ARE IMPORTANT DETERMINANTS OF OSTEOPOROSIS IN PEDIATRIC ALL PATIENTS**

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**Background.** Corticosteroids and methotrexate have adverse effects on growth and bone mineralisation. Aim and methods. The influence of single nucleotide polymorphism’s (SNPs) in the vitamin D receptor gene (VDR; 5’ Cdx-2/GATA and 3’ BsmI, ApaI, TaqI), methylenetetrahydrofolate reductase gene (MTHFR, C677T and A1298C), methionine synthase reductase gene (MTR; A66G), estrogen receptor gene (ER; PvuII/XbaI), glucocorticoid receptor gene (GR; Bcll) and collagen type 1 gene (COL1A1; Sp1 binding site), on bone mineral (apparent) density (BMD) of lumbar spine (LS) and total body (TB) were measured using DXA-scan four times during therapy and one year after therapy and expressed as standard deviation scores (SDS). Results. ER, COL1A1 and GR did not influence BMD. Carriers of the MTHFR 677 T-allele had a lower BMD-TB as compared to non-carriers after 32 weeks (DSDS 0.92, p<0.01) and 1 year of therapy (DSDS 0.95, p<0.01). The MTHFR 1298 A>C SNP did not effect BMD values. Carriers of the MTRR 66 G-allele had a lower total body BMD during therapy as compared to non-carriers (DSDS 0.72, p<0.05). Carriers of both MTHFR 677T and MTRR 66G showed a decreased BMD-TB during treatment. Carriers of haplotype 8 of the VDR 5’ Cdx-2/GATA polymorphism had a lower BMD-LS and/or BMD compared with non-carriers after 32 weeks (DSDS 0.61, p<0.05 and DSDS 0.69, p<0.05), 1 year (DSDS 0.70, p<0.05 (BMD-LS)), 2 years of therapy (_LS_1.15, p<0.01 and DSDS 1.18, p<0.01) and 1 year after cessation of therapy (DSDS 0.74, p<0.05 (BMDAD)). Conclusion. No correlations were found between fracture risk and genotype. We identified the MTHFR C677T, MTRR A66G and VDR Cdx-2/GATA SNP’s as determinants of treatment-related osteoporosis in pediatric patients with ALL.

**0982**

**LATE RELAPSES IN T-ALL PATIENTS TRUE DISEASE RECURRENCE OR SECOND T-ALL?**


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The vast majority of relapses in T-cell acute lymphoblastic leukemia (T-ALL) patients occurs relatively early, usually within 2 years from diagnosis, frequently during maintenance treatment. Our previous comparative molecular analyses between diagnosis and relapse of such ‘classical’ T-ALL (26 patients) showed totally (62%) or at least partly (58%) identical T-cell receptor (TCR) gene rearrangement patterns at both disease phases. These results confirm that the relapse clone in these patients originated from the original diagnosis clone, which became resistant to treatment-related mechanisms in this compartment, rather than to loss of CD20 expression. In vitro limited responses in the bone marrow and spleen were detected in bone marrow (mean 61±29.2%), Flow cytometric analysis of leukemic cells in periodically taken blood samples. Upon emergence of leukemic cells, the treated group received four induction doses of 250 µg RTX with 24-hour intervals, followed by three weekly maintenance doses of 250µg RTX starting 7 days after induction. Control treated animals received a control antibody directed against CD25 which was not expressed by the pB-ALL cells. During treatment, periodical monitoring of blood samples was continued. Induction resulted in complete responses (CR) in the blood of all RTX-treated animals and these CR were maintained throughout the treatment period. In the control group, administration of the control antibody did not affect leukemic progression, nor did RTX affect leukemic progression in animals engrafted with CD20- pB-ALL cells. Significant plasma concentrations of RTX (range: 4.8 to 150µg/mL) could be detected in plasma of treated animals throughout the treatment period. At experimental end-point, animals were sacrificed and blood, spleen and bone marrow were analyzed for the presence of leukemic cells. In animals engrafted with CD20- pB-ALL cells and in all animals treated with control antibody, extensive infiltration of leukemic cells was observed (mean 65±12.8%, 35±1.8% and 77±12.9% in blood, spleen and marrow of all animals, respectively). In RTX-treated animals engrafted with CD20+ leukemia no leukemic cells could be detected in blood and spleen. However, infiltrates of leukemic cells were detected in bone marrow (mean 61±29.2%). Flow cytometric analysis revealed that these cells were saturated with RTX, indicating adequate in vivo exposure. The cells expressed lower levels of CD20 as compared to cells recovered from control treated animals but were still susceptible to RTX-induced CDC in vitro. Limited responses in the bone marrow may therefore have been due to limited potential of effector mechanisms in this compartment, rather than to loss of CD20 expression. In conclusion, our results suggest that RTX may have significant activity in CD20- pB-ALL and clinical studies on the activity of RTX in CD20 positive pB-ALL are warranted.
0983

ETV6/RUNX1 DIRECTLY DYSREGULATES GENES WITH RUNX1 BINDING SITE VIA MECHANISM REVERSIBLE BY HISTONE DEACETYLASE INHIBITORS

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RUNX1 is implicated in over 30 different translocations in human acute leukemia. RUNX1, can either activate or repress transcription of key regulators of cell growth and differentiation through binding to promoters or enhancer elements. The ETV6/RUNX1 chromosomal translocation is the most common chromosomal aberration in paediatric cancers (25% of ALL). The ETV6 part of the fusion protein contains domains interacting with the mSin3, N-CoR and HDAC-3 corepressors. A part of the RUNX1 gene involved in the fusion carries DNA-binding domain. RUNX1 regulates hematopoietic myeloid cell differentiation and transcriptional activation but the role in lymphoid development is not yet fully understood. We hypothesize that ETV6/RUNX1 causes pathological differentiation block in lymphoid cells. In the current project, we utilized treatment with histone deacetylase inhibitors (HDAc). We have previously confirmed specific effect of HDACi (valproate-VPA, Trichostatin A-TSA) on ETV6/RUNX1 leukemia cells in comparison with lymphoblastic leukemias with different mechanism of leukemogenesis (BCRABL and PDGFRα/ETV6). To prove the direct effect of HDACi on ETV6/RUNX1 in vitro, we utilized a target gene of RUNX1, granzyme B (GZMB). To determine whether ETV6/RUNX1 represses GZMB via direct interaction with RUNX1-binding site at GZMB promoter, luciferase activity was measured in HeLa cells transfected with pD-NA3.1-ETV6/RUNX1/Myc and compared with HeLa with pcDNA3.1 empty vector. Cells were transfected with pGZMB-luc or pGL3-basic to normalize the luciferase activity (pGZMB-luc/pGL3-basic). Fold change of −3 FRU indicated that GZMB was downregulated by ETV6/RUNX1.

To test the direct effect of HDACi on ETV6/RUNX1, after incubation of HeLa cells with VPA and TSA, luciferase activity was monitored again. Repression activity was reduced in treated transfected HeLa cells to 53% after VPA administration and 49% after TSA administration when compared to untreated cells. We used effect of HDACi on ETV6/RUNX1 leukemia cells and identified ETV6/RUNX1 target genes in lymphoid cells. Analysis of expression profile of treated cells (VPA, TSA) vs untreated (control) ETV6/RUNX1[+] REH cells showed genes with significantly changed expression after HDACi treatment. This group of genes was compared with a group of genes associated with ETV6/RUNX1 phenotype selected by meta-analysis of expression data of ALL patients. Microarray data of selected genes showed downregulation of JunD, ACK1, PDGFRB in ETV6/RUNX1[+] patients as well as in our cell line model with increased expression after HDACi treatment. TCF4 gene was upregulated in the studied group and the administration of HDACi lead to its downregulation. Expression levels of chosen genes were validated by qRT-PCR: JunD - TSA p=0.015, VPA p=0.0008, PDGFRB - TSA p=0.0001, VPA p=0.016, TCF4 - TSA p=0.0001, VPA p=0.0002, ACK1 - VPA p=0.07. Selected genes have a fundamental role in cell proliferation and cell cycle progression therefore their role in leukemogenesis is presumptive. We show for the first time direct transcription repression by ETV6/RUNX1 on GZMB gene model. These data also support our hypothesis that HDACi affect ETV6/RUNX1[+] cells via direct interaction with ETV6/RUNX1 protein, and that treatment with HDACi may release pathological differentiation block caused by ETV6/RUNX1 aberrant transcription factor.

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0984

MINIMAL RESIDUAL DISEASE STATUS IS THE MOST IMPORTANT PREDICTIVE FACTOR IN ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA. PALG 4-2002 PROSPECTIVE ALL-MRD STUDY

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Current therapeutic protocols for adult acute lymphoblastic leukemia (ALL) take into account the risk of relapse, in order adjust the treatment intensity to individual patient needs. It is postulated that in addition to classical risk criteria including age, cytogenetics, immunophenotype, and tumor burden, also minimal residual disease (MRD) should be considered for treatment decisions. The aim of this prospective study was to evaluate the feasibility and prognostic significance of MRD detected with the use of immunophenotyping for disease-free survival (DFS) of ALL patients treated according to 4-2002 protocol of the Polish Adult Leukemia Group (PALG). Induction therapy included prednisolone, asparaginase and 4x epirubicin+vincristine. Consolidation consisted of 2x high-dose AraC+cytosphophamide, 2x methotrexate+etoposide, mercaptopurine, and CNS prophylaxis including irradiation. Patients stratified to high risk group according ‘classical’ criteria based on those formerly developed by GMALL (bcr/abl(+), WBC>30 G/L, prepreB or preT phenotype; age>35 years, or 2 courses of induction required to achieve CR) were further referred for bone marrow transplantation, whereas those assigned to standard risk group (none of the above factors present) were treated with maintenance for two years. MRD was tested at the level of 0.1% after completion of induction and consolidation therapy in patients achieving CR, employing multicolor flow-cytometry. For patients with specific antigen combinations a standard quadrans method was used, for the remaining ones we applied a new empty spaces method taking into account an individual antigen expression on blast cells. The forbidden gates were established with the use of triple staining by comparison with the pattern obtained for healthy volunteer bone marrow donors. At least two antigen combinations were tested for each patient. One-hundred–ten ALL patients (B-lineage 52%, T-lineage 17%), aged 50 years (17-61) treated in 16 hematological centers were included in the analysis. CR rate equaled 80%. Among patients who achieved CR, 24% were assigned to standard risk, 76% to high risk group, according to classical criteria. MRD evaluation was possible in all CR patients. In 50% of patients MRD was negative after both induction and consolidation - MRD(-) group, whereas in the remaining 50% of cases MRD was detected at least once - MRD(+) group. At 8 years the probability of DFS in MRD(-) and MRD(+) group equaled 58% and 28%, respectively (p=0.04). The prognostic value of MRD status for DFS was more pronounced in patients with standard risk ALL: 80% for MRD(-) vs. 0% for MRD(+) (p=0.048), than in those with high risk ALL: 51% vs. 33%, respectively (p=0.23). In a multivariate analysis including classical prognostic criteria the MRD status remained the only significant predictive factor (HR: 1.33 (1.24-22.46), p=0.04). We conclude that immunophenotyping employing empty spaces method is feasible for MRD evaluation in adults with ALL. MRD status after induction and consolidation is the most important predictive factor for DFS. In particular, patients assigned to standard risk according to classical criteria can be further stratified and those with MRD detected after induction and/or consolidation should be offered intensified treatment with the use of hematopoietic cell transplantation.
Hodgkin’s Lymphoma - Clinical Trials

0985
RECENT INTERIM ANALYSIS OF THE HD11 TRIAL OF THE GHSG: INTENSIFICATION OF CHEMOTHERAPY AND REDUCTION OF RADIATION DOSE IN EARLY UNFAVOURABLE STAGE HODGKIN’S LYMPHOMA


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Backgrounds. Combined modality treatment consisting of chemotherapy (CT) followed by involved field radiotherapy (IF-RT) is the standard treatment for early unfavourable Hodgkin’s lymphoma (HL). Despite high complete remission (CR) rates, failures are common. We thus compared the baseline-dose BEACOPP regimen with ABVD and 20 with 30 Gy IF-RT in a prospectively randomised trial (HD11) in an attempt to improve outcome in this group of patients. Methods. Between May 1998 and January 2003, 1570 patients (pts) aged 16-75 with untreated intermediate stage HL (CS I, IIA with risk factors or IIB with elevated ESR and/or ≥ 3 nodal areas only) were randomized according to a factorial design between 4 cycles of ABVD followed by 30 Gy IF-RT (arm A - standard treatment), 4 ABVD + 20 Gy IF-RT (arm B), 4 baseline-dose BEACOPP + 30 Gy IF-RT (arm C) and 4 baseline-dose BEACOPP + 20 Gy IF-RT (arm D). Results. In the fifth preplanned interim analysis, 1293 pts were evaluable for the chemotherapy comparison and 1274 for the radiotherapy comparison. Patient characteristics were well balanced between the treatment arms. 95% of patients treated reached CR, 2% had progressive disease, 8% relapsed and the total mortality rate was 4% with no significant differences between treatment arms for either endpoint. The most frequent haematological toxicities during chemotherapy were leucopenia observed in 32% of pts (ABVD: 25%, BEACOPP: 39%) and anaemia in 4% of pts (ABVD <1%, BEACOPP 7%). Infection rate was 5% (ABVD 3%, BEACOPP 7%). The most frequent toxicity during radiotherapy was dysphagia in 5%. 14 secondary neoplasias were observed: 2 AML, 4 NHL, 8 solid tumors without a significant differences between treatment arms. After a median observation time of three years, freedom from treatment failure (FFTF) was 87% (95%-CI 85-89) and overall survival (OS) was 96% (95%-CI 95-97). Both for FFTF and OS, there was no sequential significant difference either between ABVD (FFTF 87%, OS 97%) and BEACOPP (FFTF 88%, OS 96%) nor 30 Gy (FFTF 90%, OS 97%) and 20 Gy IF-RT (FFTF 87%, OS 97%). Conclusions. At three years of median observation time, no sequential significant differences in treatment outcome were detected, neither between chemotherapy regimens nor between the different doses of radiotherapy, despite more relapses in 20 Gy radiotherapy arms.

0986
COMBINED MODALITY TREATMENT OF TWO OR FOUR CYCLES OF ABVD FOLLOWED BY INVOLVED FIELD RADIOTHERAPY IN THE TREATMENT OF PATIENTS WITH EARLY STAGE HODGKIN’S LYMPHOMA: UPDATE INTERIM ANALYSIS OF THE RANDOMISED HD10 STUDY OF THE GERMAN HODGKIN STUDY GRO


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Background and Aim. Combined modality treatment is regarded as standard by most study groups for patients with early-stage Hodgkin’s lymphoma (HL). However, the optimal chemotherapy, the number of cycles needed and the optimal radiotherapy dose is still unclear. The GHSG thus conducted a randomised study for patients with early stage favourable Hodgkin’s lymphoma (HD10) in which these questions were addressed. Methods. A total of 1370 patients were randomised from 5/1998 to 1/2003 between two or four cycles of ABVD and independently to 20 Gy or 30 Gy involved field (IF) radiotherapy. Results. For the second interim analysis at a median follow up of 28 months, 847 patients were available. Patients were equally balanced for age, gender, stage, histology, performance status and risk factors. Compared with two cycles, there was more toxicity in patients receiving four cycles of ABVD for leucopenia, hair loss and infections. Concerning radiotherapy dose, there was more toxicity associated with 30 Gy for dysphagia, mucositis and leukopenia. The rate of complete remissions ranged between 98% and 99% with no significant differences among treatment arms. Freedom from treatment failure (FFTF) and overall survival showed no differences between the four treatment arms. The curves for overall survival and FFTF were nearly superimposable for all four arms. Conclusion. This analysis suggests that 2 chemotherapy cycles with involved field radiotherapy may be sufficient for patients with early favourable HL, but a reliable assessment must await the final analysis including all randomised patients and with adequate follow-up. The results of the third interim analysis (10/2005) including (10/2005) including 10 patients with a median follow up of more than 3 years will be presented.

0987
PREDICTIVE VALUE ON TREATMENT OUTCOME OF EARLY [F]-FDG PET SCAN IN ADVANCED STAGE HODGKIN DISEASE TREATED BY CONVENTIONAL CHEMOTHERAPY IS SUPERIOR TO IPS SCORE

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Backgrounds. FDG-PET scan performed early during therapy (ChT) is a powerful prognostic tool in lymphomas. Aims. Starting in January 2002, 132 new, advanced-stage HD pts, consecutively admitted to twelve Italian hematological institutions were enrolled in a prospective multi-center clinical trial aimed at comparing the predictive value on treatment outcome of International Prognostic Score (IPS) with FDG-PET scan performed after two courses of ABVD in untreated advanced stage HD patients (pts). Patients. The mean age was 33.6 years (14-79), the male to female ratio 65/67; advanced disease (stages IIB-IVB) was present in 94, and stage IIA with adverse prognostic factor (> 3 nodal sites involved, sub-diaphragmatic presentation, bulky disease and ESR > 40) in 38. Bulky and extra-nodal disease were recorded in 47 and 40 pts, respectively. All pts were staged at baseline, after 2 courses of ChT and at the end of treatment by CT scan and FDG-PET scan (CT0, PET-0; CT1, PET-1; CT2, PET-2 and CT-6, PET-6, respectively). The mean interval between the end of the second ChT course and PET-2 was 11.6 days (3-32); the interval between the end of the therapy (including radiotherapy) and PET-6 was never shorter than 50 days. 126/132 pts were treated with ABVD x 6; 6 by COPP/EBV/CAD x 6. At the end of ChT in 66/132 pts with bulky disease consolidation radiotherapy was given. All patients were given the therapy programmed at baseline, except in case of overt progression. Results. The mean follow-ups from the diagnosis and from final restaging were 609 days (73-1513) and 402 days (6-1240), respectively. 108 pts attained CR while 24 were chemoresistant: 19 showed disease progression during therapy 1 was RP and 4 showed early relapse (within 6 months) after CR entry: (+28 - +178 days). One out of the 108 pts attaining CR showed a late relapse 10 months after CR entry. In univariate analysis, besides PET-2 (p<0.01), the clinical factors that were significantly associated with a higher probability of treatment failure were stage (p<0.01), International Prognostic Score (p<0.01), WBC (p<0.01), Extra-nodal sites (p<0.01). The only factor independently significant for relapse/progression probability in multivariate analysis was PET-2, with a very high hazard ratio (80.9; 95% CI 17.9 - 207.0). In terms of treatment failure, the Positive Predictive Value (PPV) of a PET-2 and IPS (Score 0-2 vs 3 or more) were 88% and 41% and the Negative Predictive Value (NPV) were 98% and 88%, respectively. The sensitivity of PET-2 and IPS was 92% and 46%, the specificity were 97% and 85% and the overall accuracy 96% and 78%, respectively. The 2-y IPS and DDS probability for PET-2 negative and for PET-2 positive patients were 97% and 97% and 7% and 18%, respectively (FSS Log rank test = 135.1, p<0.01; DDS rank test=114.0, p<0.01). Conclusions. PET-2 scan is the most powerful tool so far available for predicting treatment outcome in advanced-stage HD.
AMH AND INHIBIN B ARE VALUABLE NEW MARKERS FOR GONADAL DAMAGE AFTER THE TREATMENT OF M. HODGKIN WITHOUT RADIOTHERAPY


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Background. An important long-term effect of both radiotherapy and chemotherapy is gonadal dysfunction. Aim of this study is to evaluate the gonadal long-term effects of the treatment for childhood M. Hodgkin (HD) with combination chemotherapy (ABVD or EBVD with/without MOPP) and to identify markers for long-term follow-up of gonadal function. Methods. Eighty-six pediatric HD patients treated from 1974-1998 were included. All patients were in complete remission. Median follow-up was 15.5 yr. (range 5.6-32.0 yr), median age at follow-up was 27.0 yr. (range 17.7-42.6 yr). Follicle stimulating hormone (FSH), luteinizing hormone (LH) and inhibin B were determined in all patients. Additionally, in men testosterone and sex hormone binding globuline (SHBG) and in women β-estradiol and anti-Müllerian hormone (AMH) were determined. In 20 men semenanalyses were performed. Results. In men treated with MOPP median FSH (16.6 U/l vs. 2.4 U/l; p<0.001) and LH (5.7 U/l vs. 2.5 U/l; p<0.001) were significantly increased as compared to patients treated without MOPP. Inhibin B (17.5 ng/l vs. 143 ng/l; p<0.001) and semen concentration (1.1*10^6/mL vs. 49.5*10^6/mL; p<0.05) were significantly decreased. Inhibin B was strongly correlated with semen concentration (rs=0.83; p<0.001). FSH (rs=0.68; p<0.001) and inhibin B (rs=-0.68; p<0.001) were correlated with cumulative dose procarbazine. In women no significant differences in LH, FSH, inhibin B or estradiol between patients treated or without MOPP were found, but AMH was significantly lower in patients treated with MOPP as compared to patients treated without MOPP (0.39 µg/L vs. 1.40 µg/L; p<0.01). AMH levels were correlated with cumulative dose procarbazine (rs=0.54; p<0.01). Conclusion. This study shows that AMH and inhibin B are valuable new serum markers for gonadal damage after pediatric HD. In men inhibin B is strongly correlated with semen concentration, whereas in women AMH detects early gonadal damage even in cases with normal LH/FSH levels.

THE POLYMORPHISM IN THE INTERLEUKIN-10 GENE PROMOTOR AT -592 IS A PROGNOSTIC MARKER IN HODGKIN’S LYMPHOMA


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Background. Hodgkin’s lymphoma is characterized by an abundant immune infiltrate surrounding the malignant Reed-Sternberg cells, and it is thought that the production of cytokines contributes to this abnormal immune response. Single nucleotide polymorphism in the promoter region of cytokine genes are key factors for cytokine production and may modify the biology of the disease. Recently, differences in the prognosis according to the Interleukin-10 (IL-10) genotype have been shown in patients with diffuse large B cell lymphomas (Lech-Miranda et al, Blood 2004; 103-3529; Aim. To assess the role of polymorphisms in the Interleukin-10 gene on progression-free survival in Hodgkin’s lymphoma. Methods. We assessed the distribution of frequencies of polymorphic allele variants in the IL-10 gene (T-3857A; G-2849A, C-2763A, A-1082G and C-592A) in 204 patients with Hodgkin’s lymphoma and analysed for association with patient characteristics and prognosis. The polymorphism were analyzed using a multiplex amplification and mismatched polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP). DNA was extracted either from peripheral blood or paraffin-embedded lymph node biopsies from 204 patients with Hodgkin’s lymphoma (median age 32 years, range 14-77 years; 91 females and 113 males). 194 patients were treated with standard chemotherapy regimen; 115 patients received ABVD, 34 pts a modified Stanford V regimen (substituting 6 mg/m² metchlomarone with 650 mg/m² cyclophosphamide), 24 pts MOPP (+ABVD), 21 pts BEACOPP. The prognostic role of allelic variants were analyzed as SNPs, and of haplotypes which were reconstructed using the PHASE programme. Results. The distribution of allele frequencies in Hodgkin’s lymphoma at position -592 of the IL-10 gene was as follows: 46% were homozygous for the CC genotype, 40% were heterozygous and 14% were homozygous for the AA genotype. The IL10 -592AA genotype was associated with a decreased progression-free survival (p=0.0074). The probability of progression-free survival at a median time of observation of 4 years for patients homozygous for the IL-10 -592 AA genotype was 35% (95% C.I, 14-54%), while for heterozygous patients and for patients homozygous for the -592 C allele it was 70 and 74% (95% C.I., 56 -80, and 61-83), respectively. When the analysis was restricted to 115 patients treated with ABVD chemotherapy, essentially the same differences in progression-free survival were observed. In univariate analysis of established prognostic factors, stage proved to be of prognostic value in our patient group (limited disease in stage I-IIA vs advanced disease in stage IIb-IV, p=0.013) The Cox multivariate analysis showed that IL-10 -592AA genotype and stage were independent prognostic factors (p=0.02 and 0.016, respectively). Conclusion. Our study indicates that the IL-10 genotype can predict clinical outcome in patients with Hodgkin’s lymphoma and points to the importance of the genetic background of the host.
**Cell signaling, transcriptional control and apoptosis - II**

**0990**

**CD95L EXPRESSING ANTIGEN-PRESENTING CELLS PREVENT ANTIGEN-SPECIFIC T CELL RESPONSE BY APOPTOSIS INDUCTION AND INHIBITION OF CTL ACTIVATION**

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**Background.** Selective depletion of antigen-specific T cells e.g. through induction of apoptosis via the CD95 system may have widespread applications in transplantation settings or in the treatment of autoimmune diseases. Antigen presenting cells (APC) expressing death-inducing ligands such as CD95 ligand (CD95L) could theoretically be used as immunomodulators in an antigen-specific counterattack system. Since human naive T cells are resistant to CD95-mediated apoptosis and acquire CD95-sensitivity only after activation, CD95L expressing APC might selectively deplete antigen-specific T cells while leaving naive T cells untouched. Aims. We studied the modulation of an alloimmune response and changes in T cell activation in the presence of CD95L-expressing APC. Methods. The HLA-A1 expressing lymphoblastoid cell line C1R.A1 was transfected with membrane-bound CD95L (m-CD95L), which was stably expressed on the cell surface of the APC due to a mutation in the metalloproteinase cleavage site. HLA-A1 negative T cells were stimulated with m-CD95L expressing C1R.A1 cells or with a mock transfectant to study the development of the HLA-A1 specific alloimmune response. Results. m-CD95L expressing APC were able to induce apoptosis in CD95 expressing activated primary T cells. Constitutive presence of m-CD95L in the stimulation cultures inhibited the development of CD4+ and CD8+ HLA-A1-specific T cells. However, immunity towards third-party, viral, and bacterial antigens was maintained and T cells spared from depletion could be induced to develop cytotoxicity towards unrelated antigens. Interestingly, inhibition of HLA-A1 specific T cell response absolutely requires the co-expression of m-CD95L and HLA-A1 antigen on the same APC. The simultaneous analysis of proliferation and apoptosis induction in HLA-A1 negative T cells activated with m-CD95L expressing APC indicated that activated T cells are depleted by cell death induction while proliferation of naive T cells was inhibited. naive T cells activated by m-CD95L expressing APC exhibited a reduced expression of activation markers (CD25, CD69, CD71, HLA CII) and Th1 and Th2 cytokines. Ca influx was diminished when cells were stimulated by CD95L expressing APC compared to the mock transfectant. However, differences in NF-kB activation were not observed independent whether m-CD95L was absent or present. Efficiency of inhibition of T cell activation by m-CD95L expressing APC was dependent on the expression level of m-CD95L. Conclusions. m-CD95L expressing APC represent efficient immunomodulators to achieve antigen-specific tolerance since they simultaneously induce apoptosis in activated T cells and prevent T cell activation of naive T cells without impairing immune responses towards unrelated antigens.

**0991**

**THE PROTO-Oncogene EVI1 INDUCES FETAL ANEMIA IN A CONDITIONAL TRANSGENIC MOUSE MODEL**

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**Background.** Aberrant expression of the proto-oncogene EVI1 has been observed in patients with acute myeloid leukemia, chronic myeloid leukemia or myelodysplastic syndrome carrying 3q26 aberrations. Patients with high EVI1 expression respond poorly to anti-leukemic therapy. Although it is generally believed that EVI1 transforms hematopoietic stem cells, there is evidence that EVI1 may interfere more directly with myeloid or erythroid development, and lineage-specific effects may play a role in the pathogenesis of leukemia or myelodysplastic syndromes. Aims. Since MDS with 3q26 abnormalities and aberrant EVI1 expression is characterized by severe anemia, our aim was to determine the direct effects of EVI1 on erythropoiesis in vivo. Moreover, our approach allowed us to investigate the effects of EVI1 when expressed at different stages of erythroid differentiation. Methods. To prevent the embryonic lethality associated with conventional EVI1 transgenic models and to allow the study of effects of EVI1 in separate hematopoietic lineages, we established transgenic mouse lines with conditional, Cre-inducible hematopoietic expression of EVI1. In these Vav-LSL-EVI1 transgenic mice, EVI1 expression is blocked by a loxP-flanked transcriptional stop sequence (LSL). The EVI1 transgenic lines were crossed with two different erythroid lineage specific Cre transgenic lines to specifically induce EVI1 expression at different stages of erythroid differentiation. The Epor-Cre and pEve-Cre transgenic lines express Cre from the BFU-E and CFU-E stage onward, respectively. Results. Erythroid-specific EVI1 overexpression induced fetal anemia, with major defects in primitive and definitive erythropoiesis. Fetal livers from both Vav-LSL-EVI1/pEve-Cre and Vav-LSL-EVI1/Epor-Cre double transgenic animals were small, pale and contained decreased cell numbers as compared to livers from single transgenic or wild type litters. However, the phenotype in Vav-LSL-EVI1/Epor-Cre embryos was clearly more severe. Colony assays demonstrated that Vav-LSL-EVI1/Epor-Cre transgenic fetal livers contained less BFU-E and CFU-E erythroid progenitors, while in Vav-LSL-EVI1/pEve-Cre embryos only CFU-E numbers were reduced. Moreover, a more complete block in terminal erythroid differentiation and a more profound increase in the number of BFU-E and CFU-E fetal liver erythroid cells were observed in Vav-LSL-EVI1/Epor-Cre embryonic ex-vivo experiments that suggest the EVI1-induced embryonic lethality in Vav-LSL-EVI1/Epor-Cre as opposed to Vav-LSL-EVI1/pEve-Cre mice may be due to a reduced sensitivity of Vav-LSL-EVI1/Epor-Cre erythroid cells to respond to Epo. Conclusion. Our results demonstrate that EVI1 pathistically interferes with the survival, expansion and differentiation of erythroid progenitors in vivo, and that the severity of the defects increases when EVI1 is induced at an earlier stage of erythropoiesis. We have established a conditional EVI1 transgenic mouse model that in combination with other inducible or lineage specific Cre-lines, e.g. Mx1-Cre can be applied to study the involvement of EVI1 in MDS and AML.

**0992**

**ROLE OF LYMPHOCYTE MICROENVIRONMENT IN INHIBITION OF APOPTOSIS AND ACTIVATION OF PI3-K/AKT PATHWAY AND PTEN IN B-CELLS**


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**Background.** The accumulation of the malignant B cells in chronic lymphocytic leukemia (B-CLL) appears to be due to inhibition of apoptosis and long survival of the leukemic cells. This could be due to the activation of anti-apoptotic mechanisms in the leukemic cells through their interaction with the lymphoid microenvironment. Aims. The aim of this study is to elucidate the role of the lymphoid microenvironment in activation of the anti-apoptotic PI3-K/Akt pathway and the prolactin and Prolactin receptor pathway in chronic lymphocytic leukemia. Methods. Stromal fibroblasts of bone marrow (BFM), spleen (SF) and lymph gland (LG) were used as an in vitro model for lymphoid microenvironment and to test their ability to inhibit spontaneous apoptosis of B-CLL. Pharmacological inhibitors and siRNAs against PI3-K and Akt were applied to explore the anti-apoptotic effect of this pathway in B-CLL. Results. Co-cultivation of B-CLL cells with human BME, LGF, and SF significantly inhibited apoptosis and prolonged survival of the leukemic cells in comparison to suspension cultures and to co-cultures with fibroblasts from non-lymphoid organs. Trans-well culture experiments indicated that cell-cell interaction and soluble mediators are essential for this protective effect. To explore the involvement of PI3-K/Akt pathway in the anti-apoptotic effect of stromal fibroblasts, co-cultures were performed in presence of PI3-K inhibitors (wortmannin or LY294002) or siRNAs against PI3-K and Akt1 and Akt2. These inhibitors significantly reduced the supportive effect of stromal fibroblasts on the survival of B-CLL cells. Conclusion. The data suggest that the lymphoid microenvironment might play a role in activation of the PI3-K/Akt pathway in chronic lymphocytic leukemia. Moreover, the results also demonstrate that PI3-K/Akt pathway is involved in inhibition of apoptosis in B-CLL and that the severity of the defects is increased when EVI1 is induced at an earlier stage of erythropoiesis. We have established a conditional EVI1 transgenic mouse model that in combination with other inducible or lineage specific Cre-lines, e.g. Mx1-Cre can be applied to study the involvement of EVI1 in MDS and AML.

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Drug resistance and treatment failure in acute leukemia has been attributed to apoptosis resistance in leukemia cells as defects in apoptosis signal transduction are commonly acquired during malignant transformation. However, expression analysis of apoptotic molecules with regard to clinical outcome has so far failed to identify apoptosis defects with prognostic value. Since the efficacy of apoptosis signaling is probably not sufficiently represented by the expression of apoptosis molecules alone, we developed and evaluated different assays to assess the function of apoptotic pathways in primary leukemia cells. Flow cytometric quantification of caspase activation by cleavage of the rhodamine derivative (Z-DEVD)2R revealed a broad variation in the extent of caspase-3 activity in primary pediatric B-precursor ALL cells upon cultivation in medium, which might be of prognostic relevance. Despite similar induction of cell death, a differential activation of caspase-3 by Cytarabine and Cyclophosphamide could be assessed, indicating drug specific differences in activation of apoptosis signaling. In a xenotransplant disease model for pediatric ALL, drug induced caspase activation could be quantified, demonstrating its potential use for monitoring drug efficacy in vivo. In order to test the functional integrity of a core apoptosis signaling pathway, we have developed and evaluated a method for the simultaneous measurement of two apoptogenic events in individual cells: caspase-3 activation and cytochrome c release, using conformation sensitive monoclonal antibodies. This method proved to identify deficient mitochondrial apoptosis signaling in leukemia cells overexpressing Bcl-2 by a pattern of apoptosis resistance, deficient cytochrome c reduction and partial processing of caspase-3. By combination of these techniques, we were able to analyze and, more importantly, to quantify potential defects in apoptosis signal transduction on a single cell level in patient samples cultured in vitro. We analyzed the activation of apoptosis signaling in primary leukemia cells during apoptosis induction by the physiologic stimulus of lack of survival factors in order to identify constitutive defects in apoptosis signaling in individual leukemia samples. Activation and mutual correlation of cytochrome c release and caspase-3 activation was quantified in 78 patient samples of precursor B-cell ALL. We identified a novel parameter, CRAC (Cytochrome c - Related Activation of Caspases 3) reflecting proficient or deficient cytochrome c related caspase activation in the individual patient sample with prognostic impact on treatment failure and relapse. At a median follow-up of 31 months, disease-free survival was 84 months (95% CI = 76 to 91 months) and 66 months (95% CI = 52 to 80 months) for patients with positive and negative CRAC respectively (p=0.019). CRAC may thus serve as a functionally defined risk factor for treatment stratification. Functional analysis of apoptosis signaling in primary leukemia may help to identify molecular targets for improvement of anti-leukemic treatment.

0993
ANALYSIS OF APOPTOSIS SIGNALING IN PRIMARY LEUKEMIA CELLS AND ITS IMPACT ON TREATMENT RESPONSE AND LONG TERM SURVIVAL
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VEGF REGULATES LEUKEMIA MIGRATION VIA FLT-1, INVOLVING PI3 KINASE, RHOA AND RAC1 ACTIVATION AND LIPID RAFTS/CAVEOLAE FORMATION
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Vascular endothelial growth factor (VEGF) and its receptors play a crucial role in malignancy and in disease, regulating the survival, proliferation, and migration of several cell types, such as endothelium and also leukemia cells. We previously demonstrated crucial roles for VEGFR-2 in acute myeloid leukemia, where its blockade showed clinical potential in murine models, by affecting leukemia survival and proliferation. In the present study we focused on a different VEGF receptor, FLT-1, and studied the molecular mechanisms whereby it modulates acute leukemia cell migration in response to VEGF/Placental Growth Factor (PLGF). First, we observed the formation of cell protrusions on ALL cells after VEGF/PLGF stimulation, with evidence for polymerized actin and FLT-1 co-localization (as determined by phallolidin, immunofluorescence staining, and confocal microscopy). Western blot analysis revealed that PLGF/VEGF stimulation resulted in increased RhoA and Rac1 GTPases expression. Co-treatment with LY200942 significantly decreased RhoA and Rac1 induction and cell migration by PLGF/VEGF, demonstrating this effect is modulated via Pi3 kinase. Next, we investigated the mechanisms whereby FLT-1 and actin co-localize at the cell ‘leading edge’ (protrusions), after VEGF/PLGF stimulation, and the relevance of such co-localization for cell migration. We addressed this question by impairing the formation of lipid rafts/caveolae using drugs whether to sequestering (nystatin) or depleting (methyl-β-cyclodextrin) cholesterol. Accordingly, co-treatment of leukemia cells with nystatin/methyl-β and PLGF/VEGF blocked cell migration, an effect that was associated with a decrease in FLT-1 polarization and co-localization with actin filaments. Instead, FLT-1 was now found mostly in the cell cytosol (possibly undergoing lysosomal degradation). Taken together, we hypothesize that FLT-1 localization in lipid rich membrane domains allows interaction with the actin cytoskeleton and downstream effectors, resulting in cell migration. Our data reveal for the first time some of the molecular mechanisms involved in VEGF-mediated leukemia migration, which may be crucial for determining the onset of extramedullary disease (the exit of leukemia cells from the bone marrow).
Anemia/Red blood cells

0995
COLD SHOCK DOMAIN PROTEIN A (CSDA) ACTS AS REPRESSOR FACTOR OF Β GLOBIN GENE EXPRESSION IN VIVO
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Impaired hemoglobin switching leading to persistent expression of fetal globin genes in adults (HPFH) offers great therapeutic potential for hemoglobinopathies and much effort is underway to clarify the molecular basis of this mechanism. In order to identify and study regulatory factors putatively involved in γ-globin gene expression, we examined the reticulocyte mRNAs differently expressed in three siblings presenting different levels of HbF and varied severity of β-thalassemia intermedia conditions, even though sharing the same α- and β-globin gene cluster genotypes. In fact, all of them showed the homozygous state for the βα IVS6-β (CT) mutation associated to haplotype VI chromosomes and a normal set of α-globin genes. To investigate the possible causes of the variations in γ-globin gene expression, extensive sequence analysis was performed on putative regulatory regions within the β-globin gene cluster. Results showed the same genetic background in all the siblings and excluded HPFH mutations. It was thus supposed that genetic determinants not linked to the β-globin gene cluster were responsible of the different γ-globin gene expression levels. To explore this hypothesis, the reticulocyte transcriptome was analyzed by a differential mRNA display approach, revealing several bands differentially displayed in the sample from the brother respect to his sisters. Selected bands were cloned and sequenced. A complete homology (greater than 95%) with the cDNA sequence of the cold shock domain protein A (CSDA) acting as repressor factor for several hematopoietic genes and previously reported to be able to interact with the γ-globin gene promoter4 was found for two of the clones originated from bands with increased expression in the brother. Quantitative real time PCR analysis of CSDA and γ-globin gene mRNA levels was performed on reticulocyte RNAs to confirm data obtained by differential display and revealed an inverted correlation between HbF values and CSDA mRNA levels, comparable to that found between CSDA and γ-globin gene mRNA. To analyze the role played by CSDA in regulating the expression of γ-globin genes, transient RNAi was used to elicit its knockdown in K562 cell line. Results showed a two-fold increased level of γ-globin mRNA when CSDA expression was interfered at about 40-50%. CSDA has been previously reported to interact with the -200 promoter region of the γ-globin gene where some HPFH mutations fall and a possible mechanism of trans-acting regulation of γ-globin gene expression has been proposed. Our data, in agreement with this hypothesis, provide further insights into the involvement of CSDA in the control of γ-globin gene expression. In fact, in our case, no HPFH mutations were detected but it is rather conceivable, on the basis of RNAi results, that a quantitative defect of CSDA expression may produce a significant persistence of HbF in adult life, thus suggesting possible novel targets for gene therapy in hemoglobinopathies.

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0996
ADJUVANT INTRAVENOUS THERAPY POTENTIATES EPOETIN β TREATMENT IN ANEMIC, NON IRON-DEPLETED PATIENTS WITH LYMPHOPROLIFERATIVE DISORDERS: RESULTS OF THE NIFE STUDY
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Backgrounds. Anemia is a common complication of malignancy. Inflammatory cytokines reduce erythropoietin production and cause disturbances in iron metabolism, most notably impaired iron uptake and mobilization from iron stores. This situation, named functional iron deficiency, may be one reason why only ~60% of cancer patients respond to epoetin therapy. Previous studies reporting a potentiating effect of intravenous (IV) iron to epoetin therapy in cancer patients may not have excluded iron depletion as a cause of anemia. AIMS. To assess whether adjuvant IV iron therapy potentiates epoetin β (NeoRecormon™) treatment of anemia in patients with lymphoproliferative disorders (LPD) and proven iron presence in the bone marrow. Methods. NIFE (NeoRecormon with Intravenous Iron [Fe]), an open, prospective, randomized study in anemic patients with indolent LPD not receiving chemotherapy, was performed in 15 Swedish centers. Sixty-seven patients with indolent non-Hodgkin’s lymphoma (n=19), chronic lymphocytic leukemia (n=25) or multiple myeloma (n=23) were randomized to receive either epoetin β only or both epoetin β and IV iron. Inclusion criteria were cancer-associated anemia (hemoglobin [Hb] ≥ 9 to ≤11 g/dL) and demonstration of stainable iron in a bone marrow aspirate. Major exclusion criteria were transfusion dependency, recent chemotherapy, serum ferritin >800 ng/mL or anemia from other causes. Epoetin β 30 000 IU once weekly (QW) was given subcutaneously for 16 consecutive weeks.

Dose adjustments were performed according to the label. Iron sucrose (Venoferr®) 100 mg QW IV was given from week 0 to 6, followed by 100 mg every 2 weeks. The primary efficacy parameter was change in Hb level from baseline to end of treatment (EOT). Secondary endpoints were Hb response rates ( % of patients with Hb ≥ 2 g/dL in the absence of red blood cell transfusion), dose of epoetin β and iron kinetics. All 67 randomized patients were included in the intention-to-treat (ITT) population, and 60 completed the study. Three patients received transfusion and/or chemotherapy and were not included in the per-protocol (PP) population of 57 patients. Results. There were no significant differences in key parameters between the two groups at baseline. The epoetin-plus-iron group had a significantly higher mean change in Hb level from baseline to EOT than the epoetin-only group (2.76 vs 1.56 g/dL [p=0.0002; ITT population] and 2.91 vs 1.50 g/dl [p<0.0001; PP population]). Hb response was reached earlier and in significantly more patients in both the ITT (79% vs 50%; p<0.02) and the PP (93% vs 53%; p=0.001) populations at EOT (Figure). Furthermore, a lower dose of epoetin was required in the group receiving iron compared with the group receiving epoetin alone (v=0.051). Conclusion: Compared with epoetin only, use of concomitant IV iron significantly increased Hb concentrations and the proportion of HB responders in non-iron-depleted patients with LPD and cancer-associated anemia. Moreover, a lower dose of epoetin was needed to achieve these better and quicker hematopoietic responses.

References
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Backgrounds. Red cell hemostasis is under the control of a highly sensitive negative feedback mechanism in which the glycoprotein hormone
erthropoietin (Epo) stimulates red cell production. Epo is synthesised by the kidney in response to hypoxia and the hypoxia-inducible factor (HIF) transcription complex, which consists of an α and a β subunit, regulates this process. Although both subunits are constitutively expressed, the α subunit is undetectable at the protein level due to continual targeting to the proteasome. In the presence of oxygen, members of the prolyl hydroxylase domain (PHD) group of enzymes actively hydroxylate proline 317 in the oxygen-dependent degradation (ODD) domain of HIF-1α. Upon hydroxylation the von Hippel Lindau (VHL) protein is able to associate and ubiquitylation occurs, consequently the α subunit is proteasomally degraded. Although defects in the VHL gene are characterised by red cell hyperplasia and identification of secondary causes, there remains a considerable number of cases where the defect remains elusive. Aims. To screen members of the PHD family of prolyl hydroxylases for molecular defects involving erythropoiesis and assess the impact of any mutations detected on the Epo negative feedback pathway. Methods. DNA was prepared and PCR-direct sequencing of the three members of the PHD family was performed. In vitro binding and enzymatic functional assays were performed using *in vitro* translated wild type and mutant PHD2 protein. Results. A heterozygous change of C to G at base 950 in PHD2 was detected in three erythrocytosis individuals from one family. All affected members exhibited subtly raised haematocrits with inappropriately normal Epo levels. The C950G base change was not detected in 200 normal control samples. This mutation results in loss of proline 317, located 2 amino acids away from an iron chelating residue in the active site, and replacement with arginine. The Pro317Arg mutant was found to exhibit reduced affinity for HIFα and its ability to hydroxylate HIFα was greatly impaired. Summary. In *in vitro* binding and enzymatic assays we have demonstrated that the Pro317Arg mutation would impair the function of PHD2, resulting in less HIF-1 α being hydroxylated and allowing more to escape proteosomal degradation. In addition, the mutation in PHD2 indicates that this is the main prolyl hydroxylase active in the regulation of HIF-1 α in the Epo pathway. There is now some evidence to suggest that deletion of PHD2 may play a role in the development of endometrial cancer thus raising the possibility that PHD2 may be analogous to VHL, where impaired function causes erythropoiesis while loss of function results in a cancer syndrome.

**0998**

**SELECTED IMIDS IMMUNOMODULATORY DRUGS: NEW APPROACHES TO THE REGULATION OF ERYTHROPOIESIS AND HEMOGLOBIN SYNTHESIS IN HEMOGLOBINOPATHIES**

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Sickle cell anemia (SCA) and β-thalassemia constitute a public health problem worldwide and new therapies are needed. Inhibition of hemoglobin S (HbS) polymerization is a major target for therapeutic approaches in SCA. New experimental therapies including hydroxyurea (HU) have attempted to augment the synthesis of fetal hemoglobin (HbF) and improve upon current treatment. Clinical trial results have demonstrated that lenalidomide (Revlimid®), recently approved by the FDA, reduces or even eliminates the need for red blood cell transfusions in some anemic myelodysplastic patients. We have examined whether CC-4047 and lenalidomide, two distinct IMiDs (immunomodulatory drugs), currently under evaluation for the treatment of hematological cancers could regulate erythropoiesis and hemoglobin synthesis. For this purpose, we used an *in vitro* culture model to differentiate human erythroid progenitors from bone marrow or peripheral blood CD34+ cells. We demonstrate that CC-4047 is a potent inducer of fetal hemoglobin (HbF) and synergize with hydroxyurea (HU) during erythroid differentiation of CD34 progenitors isolated from healthy and SCA donors. In addition, CC-4047 and lenalidomide modulate erythropoiesis, slowing erythroid maturation and increased proliferation of immature erythroid cells. Unlike other inducers of fetal hemoglobin such as HU, 5-aza-cytidine and butyrate, CC-4047 and lenalidomide were not cytotoxic. Gene expression profiling of erythroid differentiated cells showed that our drug regulate specific erythroid transcription factors and enzymes that participate in both hemoglobin synthesis and cell cycle and cellular differentiation. CC-4047 controls globin gene expression during erythroid differentiation by inducing sustained expression of fetal and embryonic hemoglobin synthesis. Our results support the hypothesis that CC-4047, alone or in combination with current approved therapies, can restore effective erythropoiesis and increase the ratio of fetal to adult hemoglobin. In addition, CC-4047 has the ability to inhibit TNF-α production and help to limit the inflammatory state in sickle cell patients. In conclusion, CC-4047 may represent an innovative new therapy for β-hemoglobinopathies.

**0999**

**RESPONSE OF MYOCARDIAL T2* TO ORAL DEFERASIROX MONOTHERAPY FOR 1 YEAR IN 29 PATIENTS WITH TRANSFUSION-DEPENDENT ANEMIAS: A SUBGROUP ANALYSIS**

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Background and aims. Patients with transfusion-dependent anemias and iron overload who were entered into multicentre studies on deferasirox (Exjade) at University College London Hospitals (UCLH) had myelodysplastic T2* performed at the Royal Brompton Hospital, as part of their routine monitoring. We have previously shown in 22 patients (16 β-thalassemia, 6 other chronic anemias) that treatment with deferasirox for 1 year at doses between 10 and 30 mg/kg/day is associated with an mean improvement in myocardial T2* of 6.4 ±1.76 ms, p=0.0026 (5.1ms geometric mean). (Porter et al, *Blood* 11, 3600, 2005) Patients and method. We now report a total of 29 patients who had myelodysplastic T2* assessment before and after 1 year of treatment with deferasirox in studies 107 (randomised DFO vs deferasirox in β-thalassemia) and 108 (deferasirox monotherapy in β-thalassemia or other anemias). This is possible because an additional 7 patients, initially randomised to DFO on study 107, have now received deferasirox for 1 year. With larger patients numbers, sub-analysis of trends in myocardial T2* response has been undertaken; in particular we have examined whether improvement in myocardial T2* is similar in patients with baseline myocardial T2* above or below the current reference normal range of 20ms. All means are reported as geometric means as the relationship between T2* and tissue iron has been considered to be logarithmic. Results. The geometric mean myocardial T2* in the 29 patients improved from 18.7ms (25%-75% CI, 11.7-29.2ms) at baseline to 23.0ms (CI, 15.3-37 ) (p=0.0065) after 1 year of treatment. If patients are divided into those with normal myocardial T2* values (T2*>20ms) and those with shortening of myocardial T2* (T2*<20ms) before deferasirox treatment, a significant improvement in both subgroups was observed. In the group of 15 patients who had normal myocardial values before treatment, the mean T2* improved from 30.3ms (CI, 25.5-36.3ms) to 36.9ms (CI, 31.5-44.8) within a year (p=0.006). In the group of 14 patients, who had abnormal myocardial T2* before treatment, a mean T2* improved from 11.2ms (CI, 9.6-12.4) to 15.9ms (CI, 10.11-16.3) (p=0.019) after one year of treatment with deferasirox. After 2 years of treatment with deferasirox only 15 patients are as yet available for baseline, 1-year and 2-year follow up analysis. In these patients, the mean myocardial T2* improved from 16.1ms (CI, 11.9-25.6) to 22.5ms (CI, 15.3-29.5) after 2 years treatment with deferasirox (p=0.005). Conclusion: Deferasirox is associated with an improvement in myocardial T2* after 1 year of treatment with deferasirox, in patients both with decreased baseline and normal baseline T2* values. Patients treated with deferasirox for 2 years maintained the improvement of the myocardial T2*. Prospective multicentre trials are now planned to study the effects of deferasirox on myocardial T2* further.
Non-Hodgkin's lymphoma
Chronic lymphocytic leukemia - Experimental

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NOTCH-MEDIATED ACTIVATION OF PI-3K IS NEEDED FOR LYMPHOMAGENESIS IN T CELL-SPECIFIC PTEN -/- MICE

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In the early stages of murine T cell development, cells go through two waves of proliferation: one mediated by IL-7 and stem cell factor, the other by triggering of the pre-TCR. An important downstream factor that is activated by IL-7 and the pre-TCR to positively regulate survival and proliferation is phosphatidylinositol-3 kinase (PI-3K). Active PI-3K phosphorylates phosphatidylinositol-4,5-biphosphate (PIP2) into phosphatidylinositol-3,4,5-triphosphate (PIP3), and this action is directly counteracted by Pten. Pten dephosphorylates PIP3 into PIP2. Previously we have shown that in mice before the age of 5 to 6 weeks, T cell-specific loss of Pten allows a β-like lineage thymocytes to bypass IL-7 and pre-TCR mediated signaling, demonstrating a critical role of Pten in regulation of ontogeny and growth of developing thymocytes. After 2.5 to 3 months of age T cell-specific Pten-/- mice showed the first clinical signs of T cell lymphomagenesis; all mice died within 25 weeks. Incubation of freshly isolated thymocytes or of established thymocyte cell lines from these mice with PI-3K inhibitors wortmannin or LY294002 induced a block in proliferation, and induced apoptosis. These data indicated that loss of Pten alone is not sufficient to drive survival and proliferation; activated PI-3K is still needed. To investigate which factors are involved in PI-3K activation, we crossed T cell-specific Pten-/- mice with mice that lacked IL-7 (γ common) and/or pre-TCR (CD3γ or RAG2) signaling. All resulting double and triple knockout mice developed lymphomas. Active PI-3K was still needed to ensure survival and growth, since thymocytes from these mice showed a block in proliferation and induction of apoptosis after incubation with wortmannin or LY294002. Similar results were obtained when cells were treated with the γ-secretase inhibitor IX (DAPT), which specifically blocks the Notch signaling pathway. Delta-like1-Notch signaling has been shown to phosphorylate Akt, a major downstream target of PI-3K, and Notch has also been shown to be involved T cell lymphomagenesis in mice. These data indicate that Notch signaling greatly contributes to PI-3K activation in Pten-/- lymphoma cells in vivo to ensure survival and proliferation.

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ALLELIC SILENCING AT 13Q14.3: A NOVEL ONCOGENIC MECHANISM


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Background. Genomic material from chromosomal band 13q14.3 distal to RB1 is recurrently lost in a variety of human neoplasms. Lack of point mutations in candidate tumor suppressor genes and downregulation of these genes in tumors indicates an epigenetic pathomechanism localized in the critical region. Aims. Characterization of the epigenetic tumor suppressor mechanism localized in 13q14.3. Methods. Candidate tumor suppressor genes are down regulated by more than a factor of two in tumors with loss of one copy of the critical region. In addition, the presence of large non-coding DNA genes in 13q14.3 is reminiscent of imprinted regions of the human chromosome 11. Therefore we designed candidate tumor suppressor genes for the epigenetic expression in healthy probands using single nucleotide polymorphisms and sequencing of RT-PCR products. Genotyping parents of these probands allowed allocation of the parental origin of either gene copy. In addition, we performed FISH experiments to measure replication timing of the two copies of the critical region to find out whether they are functionally different. As transcriptional activity and replication timing are effectuated by chromatin packaging, we used combined bisulphite-restriction (COBRA) analyses and bisulphite sequencing to assess DNA methylation of the critical region. Treatment of cultured cells with inhibitors of DNA methyltransferases and histone-deacetylases allowed function of the two copies of 13q14.3. In addition, we could detect monoallelic silencing of genes localized in the critical region and expression of one gene copy only. However, expression originated from either the maternal or paternal copy, excluding an imprinting mechanism. DNA methylation analyses showed one of the CpG islands of the region to be methylated. Demethylation of DNA and histone hyperacetylation induced full expression in both alleles, while methylation was not affected. Conclusions. We propose that differential replication timing represents an early epigenetic mark that distinguishes the two copies of 13q14.3, resulting in differential chromatin packaging and monoallelic expression. This has profound effects for the tumor suppressor mechanism localized in 13q14.3. Deletion of the single active copy of the region at 13q14.3, which is lost in more than 50% of CLL tumors, or point mutations only in the active gene copies will suffice for complete loss of tumor suppressor function, as the remaining gene copies are epigenetically silenced. Thus, we provide a model for the pathomechanism of 13q14.3 in CLL by the interaction of genetic lesions and epigenetic silencing.

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DYNAMIC MODIFICATIONS OF THE SURFACE B-CELL RECEPTOR LIGHT CHAIN IN CASES OF HAIRY CELL LEUKAEMIA OCCURRING AT EXTRAFOLLICULAR SITES

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Background. Ig gene analysis delineates critical features of the clonal history of a B-cell tumor. After antigen interaction, mature B-cells undergo somatic mutation of the V-genes and isotype switch events, generally in the germinal center (GC). Receptor revision by secondary recombination of the V-genes with re-expression of recombination activating genes (RAG) enzymes rarely occurs at this stage. From small series of cases, we have reported that most hairy cell leukemias (HCL) carry mutated VH-genes, with low levels of intrachromosomal heterogeneity, while a minor subset have unmutated VH-genes. Both subsets commonly have ongoing Ig isotype switch events and express activation-induced cytidine-deaminase (AID). However they lack CD27 and CD85 GC markers, and CD25, essential for lymph node entry. Aims & methods. In an expanded series of HCL (60 cases) with VH-genes available, the expressed VL (32) tumor-derived genes were evaluated to probe more fully the differentiation status of the cell of origin. Results. The majority (35/44, 79.5%) co-expressed multiple Ig isotype proteins on the HCs. From analysis of VH, VH3 family was most commonly expressed (98.1 and 99.6% homology in R1; 97.6 and 99.6% homology in R2). With significant preference of the VH4-30 and VH4-33 members (77.13-97.95% homology to germline), with low level of intrachromosomal heterogeneity, while 2 cases carried completely unmutated VH-genes. Analysis of the light chains showed preferential use of surface κ chain (34/50, 62%), consistent with secondary rearrangement. VH-genes were evaluated in 16 κ and 16 λ expressing HCL. All (16/16) κ cases used Jκ3 segment. Thirty of 32 cases carried mutated VL-genes (94.75%-99.6%) with low levels of intrachromosomal heterogeneity, while 2 cases carried completely unmutated VL-genes, reflecting heterogeneity in mutational status as for the VH-gene. Strikingly, cloning of the tumor VL revealed in-frame functional secondary rearrangements in 2/13 cases (Vk1 & Vk2 in Case R1, Vlampd1 & Vlampd2 in R2), most likely occurring in different tumor cells. Primary and secondary rearrangements showed mutations (98.1 and 99.6% homology in R1; 97.6 and 99.6% homology in R2). In both cases, RAG1 re-induction was also identified by RT-PCR and sequence verification. Both cases expressed AID transcripts, displayed intrachromosomal mutational variation in the VH and/or the VL-genes and 1/2 cases had ongoing isotype switch events. These data suggest a dynamic, on-going modification of the B-cell receptor (BCR) in HCs, including receptor revision, which occurs most likely in response to antigenic stimuli. N-glycosylation sites, commonly introduced by somatic mutation in the BCR of tumors of the GC, were not observed in the functional VH or VL-genes, to support the concept that tumor events occur outside the GC. Conclusions. These data confirm heterogeneity in the cell of origin in tumors with mutational status, with a minor subset of mutated V-genes. Restricted V-gene segment usage, and low levels of ongoing mutations with AID activated, coupled with the new observation of receptor revision and re-expression of RAG enzymes indicate that selective
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Backgrounds: Because B cell chronic lymphocytic leukemia (B-CLL) cannot be cured with current therapies, but in general has a slow progression and rather long median survival, it is considered an attractive candidate for active T cell mediated immunotherapy. However B-CLL cells have poor antigen presenting capacity because they express low levels of co-stimulatory molecules. Moreover, most immunotherapeutic strategies require knowledge of the eliciting tumor antigen and/or ex vivo manipulation of patient cells. To circumvent these drawbacks we aim to redirect existing viral immunity towards B-CLL. Previously, we have shown that in patients with B-CLL considerably expanded numbers of cytomegalovirus (CMV)-specific CD45RA+CD27−CD8+ T cells have been found that lyse autologous CMV-specific CD8+ T cells when directed against B-CLL cells coated with CMV peptide (A Kater et al. Br. J. Haematol. 2004; 126:512). Aim: To test a novel bridge antigen to redirect CMV-specific CTL to specifically target B-CLL. This targeting construct is composed of a streptavidin linked anti-CD20 single chain variable fragment (scFv) in combination with biotinylated HLA class I molecules containing CMV pp65 peptide (HLA/CMV). Methods: We evaluated CD20-HLA/CMV induced proliferation of CMV-specific CTL by CFSE staining and the induction of cytotoxic production via intracellular staining. Results: We demonstrate that the targeting complex is stable on the cell surface for 24h, and that B-CLL cells coated with this CD20-HLA/CMV complex can be lysed by autologous CMV-specific CD8+ CTL with similar efficiency as B-CLL cells directly loaded with CMV-peptide. Killing occurs at scFv CD20 concentrations of ≥100 ng/ml and HLA/CMV concentrations of ≥20 ng/ml. HLA-A2 positive B-CLL cells coated with HLA-B7/CMV complexes were only lysed by HLA-B7 positive CMV-specific CTL, whereas HLA-A2/CMV complex targeted HLA-A2 positive B-CLL cells were unaffected by HLA-B7 positive CMV specific CTL, proving HLA restriction of the killing. Furthermore, CD20-HLA/CMV complex coated B-CLL cells induce both proliferation and cytokine production (interferon γ, tumor necrosis factor α, and macrophage inflammatory protein-1β) in CMV-specific CD8+ T cells. Finally, we explored the requirements for interferon γ production by CMV specific T cells using blocking antibodies against LFA1, LFA2, CD80 and CD45. We demonstrate that immunological synapse formation around CD20-HLA/CMV is different from the synapse formation around pp65 loaded autologous MHC-I. Notably, lysis of both BCLL cells coated with CD20-HLA/CMV complex or directly loaded with CMV-peptide could only be blocked by anti-LFA1 antibodies (and not by LFA2 or CD80 blocking antibodies). This indicates that lytic synapse formation requires only a limited number of molecules. Summary/Conclusions: CD20-HLA/CMV complexes elicit both immune activation and direct cytotoxicity towards B-CLL cells. The findings of our study constitute a necessary step towards possible application of CD20-HLA/CMV complexes for immunotherapy of B cell malignancies. It is obvious that this recently recognized capacity to redirect existing antiviral immunity towards tumor cells has a utility in cancer immunotherapy far beyond CMV and B-CLL.
**Pharmacogenetics and molecular targeting**

**1005**

**PHARMACOGENETIC ANALYSIS OF POLYMORPHISMS IN CYP3A4, CYP3A5, GSTP1, GSTM1, GSTT1 AND MDR1 GENES FOR SURVIVAL AND THERAPY RELATED TOXICITY IN MULTIPLE MYELOMA**

A. Broly,1 C. Schiltuizen,1 E. Kamst,1 R.A. Raymakers,2 genes no Ras proteins are isoprenylated at a carboxyl-terminal domain; this may result in absence of the functional GTPase type I (GGTase-I), respectively. Activating mutations in Ras genes are very common in human cancers, including hematological malignancies. Thus, the Ras proteins are attractive anticancer drug targets. One strategy to block Ras signaling is to inhibit the enzymes that modify the CAAX motif. Inhibitors of Flase (FTIs) have shown efficacy in the treatment of some cancers. However, both K-Ras and N-Ras can be isoprenylated by GGTagase-I in the setting of FTI therapy. Consequently, we are also focusing on GTase-I. Several inhibitors of GTase-I (GTIs) have been synthesized: whereas all GTI compounds inhibit GTase-I activity, some cause growth arrest and others induce apoptosis and are lethal in mice. These compound-specific differences among different GTIs make it hard to understand their mechanism of action. Therefore, a thorough understanding of the impact of GTase-I deficiency is warranted. Aims. 1. To define the role of GTase-I in cell viability and proliferation; 2. To develop an in vivo model of K-Ras-induced leukemia. Methods. We have generated mice with a Cre-inducible GTase-I knockout allele (Pgt1flx). Moreover, we developed a new mouse model of leukemia, based on Cre-loxP techniques, where the expression of oncogenic K-Ras was targeted to granulocytes and monocytes. For this, we used mice with an oncogenic mutation (G12D) in the Kras locus (KrasG12D). In the absence of Cre, this K-Ras allele is silent. Induction of Cre turns on the expression of K-RasG12D. We have bred mice with the KrasG12D allele and a Cre transgene driven by the lysozyme M promoter (Lysm-Cre). In those mice, Cre expression, and subsequently K-RasG12D expression, is targeted to granulocytes and monocytes. Finally, to determine if inhibition of GTase-I would block K-Ras-induced leukemia, we have used Cre to simultaneously activate the expression of K-RasG12D and inactivate the expression of Pgt1 in the same cells. In this way, we determined if the absence of Pgt1 would prevent the development of K-Ras-induced leukemia. Results. We found that the Cre-induced knockout of Pgt1 abolished GTase-I activity and was compatible with cell viability in both fibroblasts and myeloid cells and caused proliferating fibroblasts to enter cell cycle arrest. Next, we developed a new mouse model of leukemia, where K-RasG12D expression was targeted to granulocytes and monocytes. We found that the KrasG12D/Lysm-Cre mice developed leukocytosis, splenomegaly, infiltration of cells in the liver, and daunorubicin (23 and 33 transporters respectively). Of these induced transporters, 12 transporters show an upregulation of more than 20-fold up to 850-fold for ABCA4 after exposure to mitoxantrone, and after exposure to daunorubicin the induction increases up to 2800-fold for ABCA6. Among the top 12 of highest induced genes, 8 transporters were overlapping between mitoxantrone and daunorubicin treated cells, including the known drug resistant gene, MDR1 (ABCB11). The remaining highest up regulated genes are currently not associated with drug resistance. Rapid and broad induction of ABC transporters upon exposure to anthracyclins was confirmed in primary CD34+ leukemic cells in vitro (n=2 patients). In the first patient sample 24 and 13 ABC transporters were more than two-fold up regulated after mitoxantrone and daunorubicin exposure respectively. In the second patient sample 11 and 15 ABC transporters were up regulated after exposure to mitoxantrone and daunorubicin respectively. There was a large overlap between the induced transporters after exposure to mitoxantrone and daunorubicin between patient samples and within patient samples. In the top ten of most induced genes were 7 transporters known to be involved in drug resistance, including the above mentioned 3 drug transporters induced in the KG1a cell line. Also in the patient samples the remaining transporters are currently not associated with drug resistance. These data show that short-term drug exposure rapidly induces a large range of ABC transporters in leukemic progenitor cells. These transporters known drug resistant transporters but not previously associated with drug resistance. The findings challenge the rationale of inhibition of single transporters to circumvent drug resistance of leukemic progenitors and warrant further research into the role of novel ABC transporters in chemoresistance of leukemic cells.

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**TARGETED INACTIVATION OF GERANYLGERANYLTRANSFERASE TYPE I RESUCES MICE FROM LETHALITY INDUCED BY ONCOCENIC K-RAS EXPRESSION IN GRANULOCYTES AND MONOCYTES**

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Background. Ras proteins are isoprenylated at a carboxyl-terminal CAAX motif by farnesyltransferase (FTase) and geranylgeranyltransferase type I (GGTase-I), respectively. Activation of Ras is essential for the development of K-Ras-induced leukemia. Aims. To develop a comprehensive analysis of both clinical and pharmacogenetic variables.

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**EXPOSURE OF LEUKEMIC CELLS TO ANTHRACYCLINES INDUCES RAPID AND BROAD UPREGULATION OF ATP BINDING CASSETTE TRANSPORTERS**

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Drug efflux by ATP-binding cassette (ABC) transporters is a well-established mechanism by which leukemic cells evade chemotherapy-induced cell death. The role of ABC transporters in chemoresistance of leukemic cells, however, remains unknown. The dynamics of ABC transporter expression in leukemic cells upon exposure to chemotherapeutic agents could identify novel transporters involved in drug resistance. We profiled gene expression of all 45 transmembrane ABC transporters after short-term drug exposure with anthracyclines (mitoxantrone and daunorubicin) in leukemic progenitor cells (KG1a cell line and primary AML CD34+ cells) by real-time RT-PCR on microfluidic cards. In KG1a cells significant induction (> 2-fold) of many ABC transporters was observed between 72 hours of exposure to both mitoxantrone and daunorubicin (23 and 33 transporters respectively). Of these induced transporters, 12 transporters show an upregulation of more than 20-fold up to 850-fold for ABCA4 after exposure to mitoxantrone, and after exposure to daunorubicin the induction increases up to 2800-fold for ABCA6. Among the top 12 of highest induced genes, 8 transporters were overlapping between mitoxantrone and daunorubicin treated cells, including the known drug resistant gene, MDR1 (ABCB11). The remaining highest up regulated genes are currently not associated with drug resistance. Rapid and broad induction of ABC transporters upon exposure to anthracyclins was confirmed in primary CD34+ leukemic cells in vitro (n=2 patients). In the first patient sample 24 and 13 ABC transporters were more than two-fold up regulated after mitoxantrone and daunorubicin exposure respectively. In the second patient sample 11 and 15 ABC transporters were up regulated after exposure to mitoxantrone and daunorubicin respectively. There was a large overlap between the induced transporters after exposure to mitoxantrone and daunorubicin between patient samples and within patient samples. In the top ten of most induced genes were 7 transporters known to be involved in drug resistance, including the above mentioned 3 drug transporters induced in the KG1a cell line. Also in the patient samples the remaining transporters are currently not associated with drug resistance. These data show that short-term drug exposure rapidly induces a large range of ABC transporters in leukemic progenitor cells. These transporters known drug resistant transporters but not previously associated with drug resistance. The findings challenge the rationale of inhibition of single transporters to circumvent drug resistance of leukemic progenitors and warrant further research into the role of novel ABC transporters in chemoresistance of leukemic cells.
and a massive recruitment of inflammatory cells in the lungs. The mice had to be sacrificed three weeks after birth. Finally, the knockout of Pgg1b rescued mice from this lethal K-Ras-induced leukemia (Figure), reduced spleen size, and the infiltration of cells in the lungs. Conclusions. Cells lacking GGTase-I enzymatic activity are viable but are unable to proliferate and the knockout of Pgg1b rescues mice from a lethal K-Ras-induced leukemia. This mouse model and genetic strategies should be valuable tools to study mechanisms and treatment of Ras-induced hematological malignancies.

1009 DEVELOPMENT OF AN EFFECTIVE SAFETY SWITCH FOR SELECTIVE ELIMINATION OF HUMAN T CELLS IN VIVO AFTER ADOPTIVE TRANSFER

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Backgrounds. Adoptive transfer of T cells is frequently associated with unwanted side-effects. In order to reduce these effects one could introduce a safety switch into the cells that permits their selective in vivo elimination. The human CD20 gene in combination with CD20 antibodies was recently proposed as a novel safety switch. In such a system, T cells may be genetically modified with a CD20-encoding vector prior to adoptive transfer. If necessary, CD20-transgenic cells can be eliminated in vivo through administration of CD20 antibodies, such as the chimeric antibody rituximab (RTX) that is currently used to treat CD20+ lymphoma cells. RTX activates the complement system and recruits immune effector cells, resulting in rapid death of CD20+ cells. Recently, a novel human CD20 antibody, Humab 7D8, was shown to have superior activity over RTX. Aims and Methods. In this study a set of CD20-encoding retroviral vectors was generated, which either lacked or contained one or both of two regulatory elements: 1) the woodchuck post-transcriptional regulatory element (WPRE) to increase CD20 expression, and 2) the chicken hypersensitivity site 4 insulator element (INS) to achieve a position independent expression of CD20 and to increase the safety profile of the vector by preventing activation of cellular (onco)genes by the retroviral enhancer. Results. We found that the level of CD20 expression obtained with vectors containing INS was 2-fold lower than with vectors lacking INS. Additional inclusion of WPRE restored the level to that of the vector without INS. In addition, INS greatly enhanced the homogeneity of CD20 expression in T cells. Moreover, after 3 months in culture, all cells generated with CD20-INS had retained CD20 expression, while 60% of cells transduced with the control CD20 vector had lost CD20 expression. Complement dependent cell kill (CDC) of both RTX and Humab 7D8 was dependent on the level of CD20 expression (p<0.01). However, while very low CD20-expressing cells were completely resistant against RTX they could be effectively killed by Humab 7D8. For maximal kill of CD20-high cells only 0.1-fold lower dose of Humab 7D8 was required, compared to RTX. In vivo efficacy was studied through bisulphimaging of luciferase+CD20-transgenic T cells. After transfer of CD20+ cells in immune deficient Rag2−/−γc−/− mice, both CD20 antibodies were capable of eliminating >99% of CD20+ cells, prolonging survival of mice from 20 till 42 days. Summary: We developed a safe vector that leads to homogeneous >99% of CD20 expression in T cells. More-over, after 3 months in culture, all cells generated with CD20-INS had retained one or both of two regulatory elements: 1) the woodchuck post-transcriptional regulatory element (WPRE) to increase CD20 expression, and 2) the chicken hypersensitivity site 4 insulator element (INS) to achieve a position independent expression of CD20 and to increase the safety profile of the vector by preventing activation of cellular (onco)genes by the retroviral enhancer.

1008 FLT3 MUTATED PEDIATRIC ACUTE MYELOID LEUKEMIA (AML) SAMPLES ARE SENSITIVE TO THE TYROSINE KINASE INHIBITOR SU11657

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Despite intensive treatment regimens only 60% of pediatric AML patients survive. Therefore novel treatment strategies to improve the outcome of pediatric AML are required. Almost 20% of pediatric AML patients harbor a FLT3 mutation (12% FLT3/ITD and 7% FLT3 D835 point mutations). Patients with a FLT3/ITD mutation have a poor prognosis. Tyrosine kinase inhibitors are novel drugs specifically targeting activated tyrosine kinases. SU11657 is one of these novel drugs and is a selective inhibitor of the tyrosine kinase receptors FLT3, KIT, PDGF and VEGF-R2. SU11657 is comparable to the currently approved SU11248 (sunitinib malate, Sutent®). In a phase I trial of sunitinib malate in AML all patients with FLT3 mutations (n = 4) had complete or partial morphologic responses compared with 2 of 10 evaluable patients with wild-type FLT3. These responses were of short duration, although longer in patients with mutated than wild type FLT3. In this study we investigated whether primary pediatric AML samples were sensitive to SU11657 in vitro and whether the effects of SU11657 are currently performing KIT mutational analysis which may explain this sensitivity in non-FLT3 mutated samples. In conclusion, there is large interpatient variation in sensitivity to SU11657. Both FLT3/ITD and FLT3 D835 positive pediatric AML samples were more sensitive to SU11657 in vitro than FLT3 negative samples.
Cancer genetics and cytogenetics in lymphoid diseases

**1010**

**MOLECULAR KARYOTYPING BY HIGH RESOLUTION ARRAY CGH UNCOVERS AMPLIFICATIONS AND HOMOZYGOUS DELETIONS IN CD138 + SELECTED PRIMARY MULTIPLE MYELOMA CASES**


Multiple Myeloma (MM) is a malignancy of clonal plasma cells with a wide variety in clinical features, responses to treatment, and survival times among patients. In 50% of the cases, the neoplastic transformation begins with a chromosomal translocation that juxtaposes the IGH gene locus to an oncogene. In addition, other genetic aberrations, as gains and/or losses of genomic regions (including trisomies and monosomies) are frequent but less characterized and they may contribute to the tumour phenotype. Our objective was to characterize copy number changes present on CD138 + multiple myeloma primary samples by means of DNA hybridization onto high resolution array CGH platforms. We conducted a high resolution analysis of copy number changes on MACS sorted CD138 + myeloma cells. Twenty-six newly diagnosed MM samples at diagnosis were included in the study. 85% of the patients carried a normal karyotype at diagnosis. The median age of our patients was 67.5 years (range: 34-85). There were 16 men and 10 women in our series. The presence of IGH rearrangement has been analyzed using SI-IGH Dual Colour, Break Apart Probe (Vysis). For molecular karyotyping Human Genome CGH Microarray 44A/B platform from Agilent Technologies (Palo Alto, CA, USA) was used for the array analysis. RESULTS. Genomic copy number analysis, performed on selected cells, allowed the identification of a high number of deletions and gains. FISH screening revealed that 9 out of 26 (35%) samples harboured an IGH rearrangement. We have discovered 267 copy number changes with a median of 8.5 changes per case, ranging from 2 to 26 copy number changes per case. By this CGH approach, we characterized whole chromosome 3, 5, 7, 9 and 15 gains in 50% of the samples. This defined the hyperdiploid group in our series. Chromosome 13 deletions have been found in 35% of the cases. Gains of chromosome 19p, 1q gain, and a novel duplication of Xq21-qter were found to be among the most frequent aberrations. In addition to big structural changes, we have also identified small rearranged regions (below 500 Kb of size). Of great genetic relevance was the finding of homozygous deletions in chromosomes 6q, 11q, 13q and Xq. The description of genomic amplifications in 6q, 9q, 16q and 17q. Finally, we have established 68 common minimal rearranged regions that will be used in unsupervised and supervised clustering. This is the first time that high resolution array CGH analysis is carried out on CD138+ on MM primary samples. This approach has allowed us to identify copy number changes in 100% of the samples and has made possible the identification of genetic relevance homozygous deletions and amplifications in MM.

**1011**

**MOLECULAR ANALYSIS OF PATIENTS WITH T-ALL USING ARRAY-CGH ENRICHED IN PROBES COVERING TYROSINE KINASE GENES**


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Background. Molecular analysis of T-cell acute lymphoblastic leukemia (T-ALL) has provided evidence for a stepwise alteration of tyrosine kinases during transformation to leukemic T-cells. Genetic alterations in hematopoietic progenitor cells lead to loss of cell cycle control, impaired differentiation, proliferation and survival advantages, and unlimited self-renewal capacity. These defects include inactivation of CDKN2A (P16) present in 96% of the patients, deregulated expression of transcription factors, and mutation of NOTCH1 present in 56% of patients. The molecular lesions leading to the proliferative and survival advantages of T-ALL cells are less well characterized and remain unknown in 80% of the T-ALLs. We have previously shown that cryptic deletions and amplifications can result in the generation of fusion genes. Examples of these are the cryptic 800 kb deletion on chromosome 9q12, and amplification of a 500 kb region of 9q34, resulting in the generation of FIP1L1-PDGFRα and NUP214-ABL1 fusion genes. Aims. Our aim was to set up a genome-wide analysis of T-ALL in order to detect cryptic deletions and amplifications, with a special focus on the 90 protein tyrosine kinase genes present in the human genome. Methods. We used the array-CGH (microarray comparative genomic hybridization) technology with slides containing genomic BAC probes spaced every 1 Mb over the human genome. An additional 480 probes were added covering the genomic locations of each of the 90 protein tyrosine kinases genes. Results. We performed array-CGH on 20 T-ALL cases. An interstitial deletion on chromosome 9p24 directly upstream of JAK2 was identified in 1 case. The deletion was confirmed by FISH. Quantitative PCR analyses indicated that the deletion was 700 kb in size including exons 1-3 of JAK2. Molecular analyses to characterize the possible presence of a fusion transcript involving JAK2 are in progress. No other rearrangements involving tyrosine kinase genes were observed in 19 other T-ALL cases, suggesting that cryptic deletions or amplifications involving tyrosine kinase genes are relatively rare in T-ALL. The most frequent aberration was the deletion of CDKN2A (14 cases). MYB duplication was found in two cases, and was confirmed by quantitative PCR. PTEN deletion was present in one case. Other unbalanced aberrations of various size were detected: del(6q) in 8/17 cases ranging from 5 to 35 Mb, del(9p) in 4/17 cases ranging from 4 to 43 Mb, dup(2q) in 2/17 cases and, dup(7q), del(7p), dup(9p) and del(12p) in one case each. Some of these rearrangements were not observed by standard cytogenetics. Conclusions. We detected a novel cryptic rearrangement of JAK2 in one T-ALL case, and duplication of MYB in two T-ALL cases. Molecular analysis of these cases, and array-CGH analysis of an additional 20 T-ALL cases and 10 T-ALL cell lines is ongoing.

**1012**

**GENE EXPRESSION PROFILING OF APOPTOSIS GENES IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA**


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The response to cytotoxic drugs varies amongst different subtypes of childhood acute lymphoblastic leukemia (ALL). We studied the expression of 70 apoptosis genes by Affymetrix U133A GeneChip microarrays in leukemic cells taken at initial diagnosis of 190 children with ALL. The expression of 44 out of these 70 genes differed significantly between T-lineage and B-lineage ALL, 22 genes differed in hyperdiploid versus non-hyperdiploid, 16 in TEL-AML1 positive versus negative, and 13 in E2A-rearranged versus negative B-lineage ALL. The data indicate that the expression of apoptosis genes highly differs between these leukemic subtypes. Expression of MCL1 and DAPK1 was significantly associated with prednisolone resistance, whereas BCL2L13, HRK and TNF were related to L-asparaginase resistance. Multivariate analysis including known risk factors revealed that BCL2L13 expression was an independent prognostic factor (p=0.011). The same trend was observed in a validation group of 92 children with ALL treated on a different protocol at the St. Jude Children’s Research Hospital (p=0.051). In conclusion, apoptosis genes are differentially expressed between subtypes of acute lymphoblastic leukemia. Out of 70 studied apoptosis genes, only 5 genes were associated with cellular drug resistance in childhood ALL. Functional studies addressing the causal relationship between these genes and drug resistance are currently being performed.
D-type cyclins are key regulators of progression through G1 phase of the cell cycle. Strong expression of at least one of the D cyclins is common in human cancers. However, while the cyclin D1 and D3 genes (CCND1 and CCND3) are recurrently involved in genomic rearrangements, especially in mantle-cell lymphoma and multiple myeloma, no clear involvement of the cyclin D2 gene (CCND2) has been reported to date in human malignancies. In T-cell acute lymphoblastic leukemia (T-ALL), the T-cell receptor genes TCRA/D and TCRA/B are frequently involved in chromosomal rearrangements and deregulate oncogenes. In order to identify new chromosomal rearrangements and oncogenes in human T-ALL, we performed an interphasic FISH screening of T-ALL cases using TCR flanking probes. By this approach, we identified two new chromosomal translocations: t(7;12)(q34;p13) and t(12;14)(p13;q11), involving the TCRA/D and TCRA/B loci, respectively. Molecular analysis of the breakpoint derivative sequences demonstrated the involvement of the CCND2 locus at 12p13. Expression analysis using RQ-PCR and immunoblotting demonstrated dramatic cyclin D2 overexpression in the translocated cases (n=3) compared to other T-ALLs (total, n=86), with a moderate expression in purified subpopulations from normal human thymus.

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Cyclin D2 expression in normal human T-cell differentiation and transition to beta-selection. In the most immature T-ALLs, a moderate CCND2 expression was observed, consistent with their differentiation stage, while low expression was found in other T-ALLs. By contrast, the maximal and sustained expression in the CCND2-rearranged T-ALL cases strongly suggested oncogenic function due to the TCR translocation. T-ALL oncogenesis is a multi-step process. We here found that the TCR-CCND2 translocations were associated with other oncogene expression (TAL1, HOXA, or TLX3/TLX1L2), NOTCH1 activating mutations, and/or CDKN2A/p16/ARF deletion, showing that cyclin D2 dysregulation could contribute to multi-event oncogenesis in various T-ALL groups. In conclusion, this report is the first clear evidence of a direct involvement of cyclin D2 in human cancer due to recurrent somatic genetic alterations. This reinforces the view that the strong expression of cyclin D2 which is detected in various types of cancer including T-ALL cases can contribute to oncogenesis, and points to cyclin D2 as a potential target for therapy in these tumors.

Mantle cell lymphoma (MCL) is a B-cell neoplasm associated with the translocation t(11;14)(q13;q32) resulting in an overexpression of cyclin D1. In addition, high numbers of secondary genomic aberrations were shown by chromosomal banding analysis, comparative genomic hybridization (CGH) and array-CGH studies. The aim of the present study was a precise delineation of chromosomal consensus regions for the most frequent genomic aberrations in MCL in order to provide a basis for the identification of candidate genes. For this purpose, a dedicated ‘MCL-array’ consisting of 4126 DNA-probes was developed. 21 genomic regions, known to be recurrently affected by genomic aberrations in MCL were covered by high resolution physical maps with a total of 3579 DNA-probes. These regions encompass: 1p13-1p22, 3q24-3q29, 6p22-6p25, 6q23-6q27, 7p15-7p22, 8p21, 8q24, 9p21-9p22, 9q21-9q22, 10p12-10p15, 11q13, 11q22-11q23, 12p12-12p13, 12q12-12q21, 13q14, 15q33-15q34, 14q32, 15q25-15q26, 17p11-17p13, 18q21-18q23, 22q11-22q13. Additionally 767 probes linearly covering the genome in a distance of 4 megabasepairs were used for the normalization of the data. A first series of cryopreserved tumor samples in 23 patients with t(11;14)-positive MCLs were analyzed. In all cases, genomic aberrations were identified. The most frequent genomic gains mapped to chromosome arm 3q (14 cases) followed by gains of 7p, 11q and 18q (7 cases each). The most frequent genomic deletion affected chromosome arm 18q (18 cases). Further deletions mapped to chromosome arms 11q (12 cases), 1p and 9p (11 cases each). The smallest consensus region for genomic gains was defined for 10p13 with a size of 600 kilobasepairs, containing the BMI1 gene. The consensus region on 8q24 was narrowed down to a size of 1.0 megabasepairs, containing the MYC gene. The smallest minimal deleted regions with a size of 600 kilobasepairs each mapped to 8p21 and 9p21, containing TNFRSF10B and CDKN2A/CDKN2B. The consensus region on 12p13 was narrowed down to a size of 1.1 megabasepairs, containing CDKN1B. These data demonstrate the usefulness of a custom made high resolution microarray as a precise tool for the delineation of genomic consensus regions in MCL. Completing a larger series of MCL these data will provide a more detailed basis for the identification of altered chromatin segments, which can contribute to the identification of candidate genes in this tumorenitivity.
Allogeneic stem cell transplantation - Clinical

1015 LOW TREATMENT-RELATED MORTALITY AND RAPID REGRESSION OF BONE Marrow FIBROSIS AFTER REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION IN PATIENTS WITH MYELOFIBROSIS. AN INTERIM ANALYSIS OF A PROSPECTIVE STUDY OF THE CHRONIC LEUKEMIA WORKING PARTY OF THE EUROPEAN GROUP OF BLOOD AND MARROW TRANSPLANTS


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Background. Allogeneic stem cell transplantation is the only curative approach for patients with myelofibrosis. Due to the high treatment related mortality only younger patients are candidate for this treatment approach. Aim. To investigate in a prospective, multicenter study the effect of a reduced-intensity conditioning regimen with busulfan (10 mg/kg), fludarabine (180 mg/m²) and anti-thymocyte globulin, followed by allogeneic stem cell transplantation in patients with myelofibrosis. Methods. At time of analysis (3/2006) 37 patients with related (n=16) or unrelated donors (n=21) were evaluable for toxicity, treatment related mortality and efficacy. According to the Lille score, myelofibrosis was classified as low (n=7), intermediate (n=22) or high risk (n=8). The median age of the patients was 53 years (range, 32-67). Stem cell source was peripheral blood stem cells (n=36) or bone marrow n=1 from HLA-matched donor (n=32) or HLA-mismatched donor (n=5). Results. No primordial failure was observed. The median time until leukocyte (>1.0x10⁹/L) and platelet (>20x10⁹/L) engraftment was 17 days and 22 days respectively. The leukocyte engraftment was faster in immature patients (p=0.0001). Acute graft-versus-host disease (GvHD) grade II IV and III/IV occurred in 21% and 8% of the cases, and 21% of the patients experienced chronic GvHD. The treatment-related mortality at one year was 6%. The cumulative incidence of relapse at three years was 16%. After a median follow-up of 12 months, the estimated three-year overall and disease-free survival was 85% and 75% respectively. The three-year estimated disease-free and overall survival was 77% and 85% respectively. The disease-free survival was higher in low risk than in intermediate/high risk patients (100 vs 70%). In 20 patients, the dynamics of bone marrow changes could be monitored one month, six months and one year after stem cell transplantation by sequential bone marrow trephine biopsies. A total regression of the pre-transplant increased fibrosis was observed in the post-transplant period after about six months while the extent of osteosclerosis did not change significantly during observation time. The CD34 progenitor cells in bone marrow (1.3x10⁶ to 1.3x10⁷) of the transplanted patients the number declined rapidly to normal values in all responding patients. Conclusions. Reduced-intensity conditioning in patients with myelofibrosis provide rapid and sustained engraftment with a low one-year treatment-related mortality resulting in an encouraging three-year overall and disease-free survival. Allogeneic stem cell transplantation results in rapid regression of fibrosis and in rapid decline of CD34 progenitor cells in the bone marrow.

1016 HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION WITHOUT IN VITRO T CELL DEPLETION FOR THE TREATMENT OF HEMATOLOGICAL MALIGNANCIES


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Backgrounds. Many patients who require allogeneic hematopoetic stem cell transplantation (allo-HSCT) do not have a human leukocyte antigen (HLA)-matched donor. HSCT from HLA-mismatched family donors is associated with lower long-term survival and delayed immune reconstitution, especially if the T cells are depleted. Aims. Here we describe a method for haploidentical allo-HSCT from family members without in vitro TCD, designed to overcome the HLA barrier and reduce transplant-related complications. Results. In the present study, the method for haploidentical allo-HSCT without in vitro TCD involves sequential, in vivo modulation of T cell functions in the recipient and the donor, and adjustment of the dose of donor HSCs. This protocol has three elements: anti-human thymocyte immunoglobulin (ATG) for the prevention of GVHD, a combination of G-PBSCs and G-BM, and donor treatment with recombinant human G-CSF: There are 176 patients, including 88 with high-risk malignancies, underwent transplantation from an HLA-haploidentical family donor with 1-3 mismatched loci. All patients achieved sustained, full donor chimerism. The cumulative incidence for acute GVHD was 22.7%, which was not associated with HLA disparity. The cumulative incidence for extensive cGVHD was 46.9%. The two-year probability of relapse was 12.2% in the standard risk group and 58.9% in high-risk group. The probability of 1-year and 2-year leukemia-free survival (LFS) was 72% and 68.2% for standard-risk patients and 54.3% and 42.1% for high risk patients (p=0.0009) respectively. Summary/Conclusion: These results show that G-BM combined with G-PBSCs from haploidentical family donors, without in vitro TCD, could be used as a good source of stem cells for allo-HSCT. The new HSCT regimen described here allows use of a haploidentical family members as donors, a strategy likely to be much more important in the future, for the increasing numbers of Chinese patients, and those of other ethnicities, who are the only child in the family.

1017 PURIFIED T DEPLETED PERIPHERAL BLOOD AND BONE MARROW CD34+ TRANSPLANTATION FROM HAPLOIDENTICAL MOTHER TO CHILD WITH THALASSEMIAS


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Approximately 60% of thalassemic patients cannot apply to ‘gene therapy today’ which the insertion of one allogenic HLA identical stem cell into the empty bone marrow as the vector of the normal gene for β-globin chain synthesis. Work today’ which the insertion of one allogenic HLA identical stem cell into the empty bone marrow as the vector of the normal gene for β-globin chain synthesis. We studied the use of haploidentical mother as the donor of hematopoietic stem cells assuming that the immunotolerence established during the pregnancy will help to bypass the HLA disparity and allow the hematopoietic allogeneic reconstitution in the thalassemic recipient of the transplant. We have employed a new preparative regimen for the transplant in fourteen thalassemic children aged 3 to 12 years (median age 5 years) using T cell depleted peripheral blood stem cell (PBSCs) plus bone marrow (BM) stem cells. All patients received hydroxyurea (OHU) 60 mg/kg and azathioprine 3 mg/kg from day -59 until day-11, fludarabine (FLU) 50 mg/m² from day -17 to day -7 and busulfan (BU) 14 mg/kg starting on day -11, fludarabine (FLU) 30 mg/m² from day -17 to day -1 and cyclophosphamide (CY) 200 mg/kg. Thiopeta 10 mg/kg and ATG Sängastat 2.5 mg/kg, followed by a CD34 + cell depleted (CliniMacs system), granulocyte colony stimulating factor (G-CSF) mobilized PBSC from their HLA haploidentical mother. The purity of CD34+ cells after MACS sorting was 98-99%, the average number of transplanted CD34+ cells was 9.9x10⁶/kg and the average number of infused peripheral blood cells (PB-C) from BM was 1.8x10⁴/kg. The patients received cyclosporin after transplant for graft versus host disease (GVHD) prophylaxis during the first two months after the bone marrow transplantation. Results. All patients are alive. Three patients rejected the transplant and are alive with thalassemia. Two patients engrafted after a second transplantation. Eleven patients are alive disease free with a median follow up of 26 months (range 7-42).None of the seven patients showed AGVHR. This preliminary study suggest that the transplantation of megadose of haploidentical CD34+ cell from the mother is a realistic therapeutic option for those thalassemic patients without genotypically or phenotypically HLA identical donor.

1018 INCREASED CD4+CD25 HIGH REGULATORY T-CELL ARE ASSOCIATED WITH DISEASE RELAPSE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION FOR CHRONIC MYELOID LEUKAEMIA (CML)

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Backgrounds. The success of SCT after CML largely relies on the graft versus leukemia (GvL) effect exerted by donor T-cells. CD4+CD25 high regulatory T-cell (Treg) play a crucial role in the maintenance of peripheral tolerance and have been tested in animal models to successfully prevent GVHD. The role of Tregs in clinical transplantation remains unclear, insofar as the few studies published to date have reported controversial results. However, the role played by Tregs in stem cell transplantation is unclear in CML...
results regarding GrVHD. Although there is emerging evidence that Tregs are associated with a poor outcome in cancer patients, none of these studies has investigated the role of Tregs in leukemia relapse post-SCT. Aims. To quantify CD4CD25high regulatory T-cells in post-SCT patients and correlate their levels with clinical outcome. Materials and Methods. We performed a cross-sectional study at a single institution. We enumerated and characterized peripheral blood CD4CD25high T-cells in 76 patients after SCT for CML by FACS analysis. Acquired defects were then analyzed in 21 samples from healthy volunteers and 20 samples from newly diagnosed CML patients. BCR-ABL/ABL ratio was determined in every sample by real-time PCR. Patients were considered in remission if the ratio was less than 0.02% and in relapse if higher. All quoted p-values are two-sided with p<0.05 considered statistically significant. Results. Patients after SCT had higher levels of Treg than normal donors (median 1.5% vs. 0.87, p<0.001) and untreated CML (median 1.5% vs 0.27, p<0.0001). In the multiple regression analysis only the time post SCT (before or after 18 months) and disease status (molecular remission versus relapse) were predictive for increased Tregs (Coef-2.994, p<0.004 and Coef-2.935, p=0.020 respectively). No association with Treg levels and GrVHD was found. The logistic regression analysis performed in 43 patients that had not received DLI post SCT confirmed that increased Tregs, both as percentage or absolute numbers, were the only predictive variable for relapse (exp 1.44, p=0.011). Conclusions. A substantial expansion of Tregs occurs early after allogeneic SCT and the presence of high numbers of Tregs 18 months after transplant is predictive of leukaemic relapse. Although the increment might initially have an advantageous effect on graft rejection, our data suggest that Tregs exert an inhibitory effect on GV.L.

1019 CHRONIC GVHD HAS A ROLE IN MAINTAINING REMISSIONS AFTER IMatinib IS DISCONTINUED IN PH-ALL PATIENTS TRANSPLANTED IN CR1
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Backgrounds. Reappearance of BCR-ABL transcripts after allogeneic stem cell transplantation for Ph-ALL indicates evolving relapse. Intervention with imatinib may be associated with renewed and sustained PCR negativity. However, the clinical consequences of discontinuing imatinib are not known. Methods. We updated a previously reported prospective study of post-transplant imatinib for molecular remission to assess the outcome of patients in whom imatinib was discontinued after sustained PCR negativity. Results. The present analysis includes exclusively the subset of 14 patients in whom BCR-ABL transcripts became undetectable shortly after (median: 46d, range: 27-111d) initiation of imatinib. Fourteen of 15 patients who did not achieve early PCR negativity had relapse. When imatinib was discontinued after 12.2 (range: 1.4-17.5) months, BCR-ABL transcripts were undetectable in 13 of the 14 patients who had achieved PCR negativity. A median of 16.5 (range 3.3-39.4) months after stopping imatinib, 6 of these 14 patients were PCR negative and alive, one experienced reappearance of BCR-ABL transcripts and is currently treated with imatinib and donor lymphocyte infusions (DLI), 3 patients died in molecular CR, and 3 patients relapsed (CNS:n=1, BM:n=2) 3.3, 16.4 and 13.6 months after imatinib discontinuation. One patient converted to PCR positivity while still on imatinib and was entered in a clinical trial of AMN107 in conjunction with DLI. None of the 8 patients with active chronic GrVHD at imatinib discontinuation relapsed either at the molecular level or hematologically, with a median follow-up of 16 (range 6.2-22.3) months. Estimated probability of remission 24 months after discontinuation of imatinib in patients with and without chronic GrVHD was 100% and 40%, respectively (p=0.04). Conclusion: We conclude that it is appropriate to discontinue imatinib in patients who previously underwent allogeneic SCT, remained PCR negative on imatinib for approximately one year, and have ongoing chronic GrVHD. Absence of GrVHD is associated with a higher risk of relapse following termination of imatinib therapy.

Platelets and bleeding disorders

<table>
<thead>
<tr>
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<th>Myeloproliferative</th>
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<tr>
<td>Case number (%)</td>
<td>122 (51)</td>
<td>118 (49)</td>
<td>240 (100)</td>
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<tr>
<td>Bleeding score (&gt; 10)</td>
<td>30/122 (25)</td>
<td>18/118 (15)</td>
<td>48/240 (20)</td>
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<td>79/240 (33)</td>
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<td>59/240 (25)</td>
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<td>2) ASPD</td>
<td>0/122 (0)</td>
<td>19/118 (16)</td>
<td>19/240 (8)</td>
</tr>
<tr>
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<td>8/122 (7)</td>
<td>3/118 (3)</td>
<td>11/240 (5)</td>
</tr>
<tr>
<td>4) anti FVIII or X inhibitors</td>
<td>3/122 (2)</td>
<td>4/118 (3)</td>
<td>7/240 (3)</td>
</tr>
</tbody>
</table>

In one year, severe mucosal (n=21) and non-mucosal (n=13) bleeds in LDF (n=12) or MPD (10) were treated with DDAVP (n=18), IFP/concentrates (n=4), IVIG (n=10), rFVIIa (n=2). Conclusions. AVWS and the other acquired hemostatic defects shown here are not so rare (9/16%) and can be severe in LDF/MPD. An early correct diagnosis should improve morbidity and mortality of patients with bleeding complications in chronic LDF/MPD.

1021 DIFFERENT ALLELIC DISTRIBUTION OF SINGLE NUCLEOTIDE POLYMORPHISMS AT CODONS 10 AND 25 OF F9B IN A GROUP OF 122 ITALIAN PATIENTS AND CONTROLS
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Hereditary hemorrhagic telangiectasia (HHT) (OMIM #178300) is an autosomal dominant vascular disorder characterized by telangiectases on skin and mucosa (causing epistases and gastrointestinal bleeding, that may be severe enough to require transfusions) and visceral arteriovenous malformations (AVMs). Epistaxes and telangiectases are the most frequent symptoms, present in more than 95% of the patients. AVMs...
are mostly observed in liver, lungs and brain and may cause severe life-threatening complications. The phenotype is highly variable, even among members of the same family, and penetrance is usually complete by the age of 40 years. About 80% of HHT patients carry mutations in either of two genes: ENG (OMIM #131195) (HHT1) or ACVR1L (OMIM #601284) (HHT2) which code for a TGFβ receptor type III and I respectively. "The 5' end of the ACVR1 gene contains many polymorphisms, mostly SNPs, some of which affect its transcription, causing individual variations in protein production. Aims: To assess the genotype distribution of TGFβ codon 10 and 25 polymorphisms (which are known to be related to protein production levels) in a group of 122 HHT patients (58 index cases) affected with HHT in whom the causing mutation in ENG or ACVR1L is known. Methods: A 500 bp fragment of TGFβ gene including codons 10 and 25 was amplified by PCR and subsequently digested by MspAI (codon 10 SNP) and FseI (codon 25 SNP) enzymes. Digested products were analysed on polyacrylamide 7% and agarose 3% gels respectively. A subgroup of 20 patients was genotyped by PCR-SSP using the cytokine typing kit provided by Pel Freez Company. Statistical analysis was performed using the χ2 test (202 controls). Results. A statistically significant difference in the distribution of codon 10 and 25 was observed; for codon 10, it was limited to the subgroup carrying the ACVR1L mutation, while in the allele distribution at codon 25 was more widely different from controls. Evidence for linkage disequilibrium and statistical differences in the TGFβ producer genotypes between controls and HHT patients: in this last group, in fact, there is a higher than expected percentage of intermediate and low producers (p<0.01). Summary: HHT is a vascular autosomal dominant disorder characterised by nosebleeding, telangiectases and AVMs. A wide inter and intra-familial variability in the phenotype is present. The genes involved (ENG and ACVR1L) belong to the TGFβ signalling pathway and we assessed genotype distribution of two TGFβ SNPs (codons 10 and 25) related to the protein production in a group of 122 HHT patients with a known causing mutation. Statistically significant results were obtained for codon 25 and some codon 10 subgroups. We suggest that differences in TGFβ production can partially explain the phenotypic variations.

1023
Eltrombopag increases platelets during 6-week treatment of ITP results of a randomized, double-blind, placebo-controlled phase II study
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Background/aims. Eltrombopag olamine, a novel, small molecule, oral platelet growth factor was studied in a global, randomized, double-blind, placebo-controlled phase II trial in adult patients with chronic idiopathic thrombocytopenic purpura (ITP) and platelet counts <30,000/µL. Methods. The primary efficacy endpoint was the proportion of patients with platelets greater than or equal to 50,000/microL after 42 days of dosing using last observation carried forward. Randomization was stratified by spleenectomy status, use of concomitant ITP therapy and platelet counts less than or equal to 15,000/µL. Results. One hundred and four patients were randomized, into placebo (N=26), 50mg (N=27), 50mg (N=27) and 75mg (N=24) eltrombopag arms. The majority of patients were females (62%) and of Caucasian origin (70%). Prior treatment of ITP included corticosteroids (73%), IVIG (37%) and anti-D (27%). During the study 35 (34%) patients received concomitant ITP therapy. At Day 45, a dose dependent increase in the proportion of responders (platelet count greater than or equal to 50,000/µL) was observed: placebo (16%), 30 mg (28%), 50 mg (67%) and 75 mg (86%). The odds-ratio of treatment response to placebo was statistically significant in the 50 and 75mg arms (p<0.001). Similar efficacy response of eltrombopag relative to placebo was observed regardless of strata (spleenectomy status, co-administration of ITP treatment and baseline platelet count). The percentage of patients achieving a platelet count >200,000/µL was: placebo (4%), 30mg (12%), 50mg (38%) and 75mg (52%). The median platelet count on Day 45 was 16, 29, 132 and 202,000/µL for placebo, 30mg, 50mg and 75mg eltrombopag, respectively. Overall, the safety profile was similar across the treatment groups, with the following percentage of patients experiencing at least one adverse event (AE) during treatment: placebo (58%), 30 mg (44%), 50 mg (44%) and 75 mg (58%). Headache, AST elevation, constipation and epistaxis were the only AEs occurring in greater than or equal to 5% of patients in the eltrombopag arms. The most common AE in the placebo arm was fatigue, occurring in 19% of patients, compared to 8% of all patients exposed to eltrombopag. The most common AE in the eltrombopag 75mg arm was headache (21%), compared to 15% of patients exposed to placebo. A total of 2/3 placebo and 2/7 50mg patients experienced at least one serious AE (SAE) during treatment; of which, 1/4 placebo patient and 1/4 50 mg patient experienced at least one related SAE during treatment. No SAEs were reported during the treatment period on the 30 mg and 75mg eltrombopag arms. No other dose dependent safety concerns were identified. Summary/conclusions. Elnrombopag at doses of 30 and 75mg significantly increased platelet counts during the 6 week treatment period compared to placebo. No dose dependent safety concerns were identified. Phase III trials of eltrombopag in patients with ITP are ongoing.
Backgrounds. Eltrombopag is a non-peptidyl small molecule thrombopoietin receptor (TpoR) agonist currently in clinical development for the treatment of patients with thrombocytopenia. Simulation of this receptor results in enhanced megakaryocyte proliferation, differentiation, and ultimately platelet production. In addition to effects on megakaryocytes, TpoR activation via thrombopoietin (Tpo) is known to directly stimulate platelet function. The physiological consequences of platelet stimulation in this setting are unclear; however, it could represent a general liability in the utilization of TpoR agonists. Aims. The objective of the present study was to compare the direct platelet activating potential of eltrombopag to Tpo in vitro. Methods. Platelets were obtained from healthy volunteers, and the activation of signal transduction pathways was examined in washed platelet preparations. Platelet aggregation was examined under multiple experimental conditions, including washed platelet preparations and platelet-rich-plasma (PRP) anticoagulated with either citrate or hirudin. Platelet α-granule release was determined via FACS measurement of CD62P. Results. In signal transduction studies of washed human platelets, Tpo activated Stats-1,3,5 and Akt. In comparison, eltrombopag partially activated Stats-3 and 5, with no/minimal activation of Stat-1 or Akt. In platelet aggregation studies, Tpo acted in synergy with subthreshold/submaximal concentrations of ADP or collagen to induce maximal aggregation under all conditions examined. In contrast, eltrombopag induced weak and inconsistent activation of washed platelets; however, no synergy was observed when examined in PRP. Similar to aggregation results, platelet activation as examined via surface expression of CD62P was significantly enhanced by Tpo as compared to eltrombopag. Conclusions. The present study demonstrates that the TpoR agonist eltrombopag has only a limited capacity to induce human platelet activation, suggesting that potential platelet activation liabilities associated with peptidyl TpoR agonists could be attenuated via a small molecule approach.
1026
RANDOMIZED COMPARISON OF IMATINIB WITH IMATINIB COMBINATION THERAPIES IN NEWLY DIAGNOSED CHRONIC MYELOGENOUS LEUKEMIA PATIENTS. DESIGN AND FIRST INTERIM ANALYSIS OF A PHASE III TRIAL


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**Backgrounds.** Imatinib (IM) at 400 mg daily has emerged as the preferred therapy for newly diagnosed CML pts. Despite impressive results, only a minority of pts treated with IM achieved a molecular remission. To improve upon these results, the CML French Group designed a phase III, multicentre, open-label, prospective randomized trial. Methods. The experimental arms are IM 400 mg daily in combination with Peg-IFN α-2a, 90 µg weekly or IM 400mg daily in combination with Ara-C, (20 mg/m²/day, days 1-28 of 28-day cycles) or IM 600mg daily. The reference arm is IM 400mg daily. All pts (over 18 years of age with Bcr-Abl positive CML in chronic phase within 3 months of diagnosis) receive IM 400 mg/day as monotherapy days 1-14 and then start the assigned randomization. Randomization continues at least 12 months or until treatment failure or major toxicity. The primary endpoint will be the overall survival. Other endpoints will be: rate and duration of hematologic and cytogenetic responses, major (McyR) and complete (CcYR), molecular response and the tolerability. Using treatment allocation ratio 1.1.1.1, randomization is stratified according to Sokal risk groups. An interim analysis of the first 636 patients (±10%) at 1 year from randomization will allow evaluation of molecular response rates, one of the experimental arm being selected for further comparison with IM 400. The increased dose of IM or a combination regimen would be considered as promising if it increased the 4 log reduction response rate by at least 15 percentage points, e.g. from 15% to 35%, with an acceptable tolerability. Evaluation of molecular response up to 12 months is centralized and blinded. Results. This evaluation is based on a cohort of 315 pts with a median time of observation of 12 months, [median age 53 yrs (18-78), 60% of pts were male; Sokal risk distribution: 39% of pts low risk, 61% intermediate risk, and 25% high risk]. At 3 months 92% of pts achieved complete hematologic response. Cytogenetic data are available from 154 pts. At 6 months, 135 pts (87%) achieved a MCyR, being complete in 105 pts (68%). Grade 3/4 neutropenia and thrombocytopenia occurred in 5% and 0% of IM400 pts , in 5% and 1% of IM600 pts, in 30% and 4% of IM+IFN pts and in 24% and 13% of IM+Ara-c pts respectively. Dose of Peg IFN was reduced in 16% of pts, 45 µg per week in 30% and 4% of IM+IFN pts and in 24% and 13% of IM+Ara-c pts (mainly diarrhea). Discontinuation of experimental treatment occurred in 5% and 0% of IM400 pts , in 5% and 1% of IM600 pts, in 5% of IM+IFN pts (mainly skin rash) and in 13% of IM+Ara-c pts (mainly diarrhea). Discontinuation of experimental treatment occurred in 15% of IM600 pts, 22% of IM+IFN pts and in 13% of IM+Ara-c pts.

Conclusions. This first analysis has proven feasibility of IM combinations in addition to high response rates. However a substantial hematologic toxicity was recorded with IFN or Ara-c combination, which requires a careful assessment during the first 6 months of treatment.

1027
A FLOW-CYTOMETRIC IMMUNOBEAD ASSAY FOR THE DETECTION OF BCR-ABL FUSION ONCOPROTEIN IN PRECURSOR B-ALL


**Erasmus MC, Rotterdam, Netherlands**

The BCR-ABL fusion gene, caused by t(9;22), is a frequent chromosomal aberration in precursor B-ALL patients (25-40% of adults cases, 5-5% of pediatric cases) and is associated with a poor prognosis, requiring a high-intensity treatment protocol. Rapid detection of the BCR-ABL translocation in precursor B-ALL patients at diagnosis is therefore important for the choice of treatment. Current approaches for the detection of the BCR-ABL fusion gene employ karyotyping, FISH or PCR. However, these techniques take relatively long and demand a specialized laboratory. Our aim was to develop a flow cytometric bead-based assay (CBA) that detects the BCR-ABL fusion gene product in an easy, rapid and accurate manner. In this assay, a bead-bound capture antibody recognizes one part of the fusion protein, whereas a biotin-conjugated detection antibody recognizes the opposite part of the fusion protein. Only when a lysate of patient cells contains the fusion protein, immunobeads will give a positive signal in the flow cytometer. We generated a novel anti- biotin detection reagent, the exon 1 encoded domain of the BCR protein, using a developmental strategy and screening method that increases the likelihood of producing antibodies that are suitable for flow cytometry. The anti-BCR antibody was coupled to BD CBA Flex beads. After incubation of these beads with cell lysate, an already existing biotinylated anti-ABL antibody was used as detection antibody together with streptavidin PE. Using this bead assay, we detected a strong PE signal in cell lysates of the cell lines K562, LAMA-84 (both p210), TOM-1 (p190) and AR230 (p230) harboring the BCR-ABL translocation, but not in cell lines with other translocations or normal PBMCs. A robust signal could be detected with less than 1% of LAMA-84 cells in a background of normal PBMCs, showing the high sensitivity of the assay. We then tested the assay on lysates of precursor-B-ALL patients and showed a highly specific signal only in patients that were tested positive for the BCR-ABL fusion gene using standard PCR and/or FISH techniques. We conclude that this novel bead assay can be used for diagnosis of precursor-B-ALL patients. The new assay has major advantages over currently used techniques. It is fast (completed within a few hours) and can easily be performed in a standard diagnostic laboratory using routine flow cytometry. The assay is accurate and sensitive and as recognition does not involve the break-point region, all different BCR-ABL protein variants (p190, p210 and p230) can be detected. Furthermore, as the assay involves BCR-ABL protein rather than the DNA, it will measure the presence of cells that are sensitive to imatinib therapy and may be used for monitoring of treatment effectiveness.
Deletions at the ABL-BCR reciprocal breakpoint on the derivative chromosome 9 are seen in 10-15% of patients with CML and have been associated with a poor prognosis, at least for cases treated with hydroxyurea or interferon-α (IFN). Studies to date have used different FISH probe sets to determine deletion status and thus the results are not always directly comparable. Furthermore, information concerning the extent of deletions is limited. To provide more accurate information about deletion status, we have developed a rapid DNA-based screen based on multiplex ligation-dependent probe amplification (MLPA). Probes were designed to the deleted region both the upstream and downstream of the breakpoint, plus several control loci. MLPA was performed using standard conditions and peak heights were determined using an ABL 3100 Genetic Analyzer and Genotypy software. Since patient samples may contain a variable proportion of normal cells, we determined the sensitivity of the assay to detect the der(9) deletion in MC3 cells. We found that the deletion was readily detectable in dilutions of MC3 DNA in normal DNA at a level of 60% or greater, indicating that the assay was applicable to the great majority of CML patients. We then went on to perform a retrospective study of 348 patients (204 male; 144 female; median age 50 years, range 11–85) who had been enrolled into the German CML I, II or III studies between 1987 and 1999. This represents the largest study on the prognosis of der(9) deletions to date. All patients received IFN as first line therapy but 61 were subsequently treated with imatinib and 138 subsequently underwent stem cell transplantation (SCT). At the time of analysis, 161 patients were still alive at a median of 8.8 years (range 2.6-16.5). MLPA was performed on samples taken prior to treatment and showed that der(9) deletions defined as loss of at least two consecutive markers. Unexpectedly, we found that patients with deletions survived longer than those without deletions, although the difference was not significant (9.2 versus 7.3 years; p=0.17). This effect was seen both for cases that underwent SCT (not deleted: n=116, median = 9.4 years versus deleted: n=22, median not reached; p=0.34) or did not undergo SCT (not deleted: n=173, median = 6.8 years versus deleted: n=87, median = 9.8 years; p=0.27). However, the 21 cases who had deletions that spanned the translocation breakpoint did show inferior overall survival when compared to all other cases (5.7 versus 8.8 years; p=0.0025). This was not seen for patients with deletions on the ABL side only (n=20) or the BCR side only (n=18) and in fact both these groups showed longer survival compared to all other patients. In conclusion, MLPA is a reliable technique for detection of der(9) deletions in CML. Our analysis indicates that only those deletions that extend to both sides of the reciprocal ABL-BCR fusion breakpoint are associated with adverse prognosis.
myelodysplastic syndrome (MDS) and 5q1 deletion. Lenalidomide has been shown to inhibit angiogenesis, cell adhesion, and secretion of TNF-α, and to modulate other cytokines. Furthermore, lenalidomide stimulates T-cells and NK-cells, and directly induces apoptosis in myeloma cells. It is not well characterized how these effects are mediated, nor which effects that are central for the impact on cancer cells. Aim: To assess the direct effects of lenalidomide on growth, differentiation, and gene expression of hematopoietic cells from MDS patients with del(5)(q31) and healthy controls. Methods: Selected CD34 hematopoietic progenitors from 12 MDS patients with del(5)(q31) and from 10 healthy controls were cultured with or without 10 µM of lenalidomide in a 14-day model for erythroblast differentiation (with medium containing IL-3, IL-6, and SCF, with addition of Epo during the second week). FISH and FACS analyses were performed at day 0, 7, and 14. The median proportion of 5q− cells by FISH at day 7 was 98% (range 86−99), dropping to 84% (range 14−98) at day 14 due to a variable outgrowth of cytogenetically normal cells. Gene expression profiling was performed on day 7 cells from 6 MDS patients and 5 healthy controls using Affymetrix Human Genome U133 Plus 2.0 Arrays. Results: In erythroblast cultures with cells from healthy controls, lenalidomide had no inhibitory effect on fold increase of cell counts (p=0.60). However, in cultures with cells from 5q− patients, the clone with 5q deletion showed significant inhibition of fold increase at day 14 (p=0.04), while the cytogenetically normal progenitors were not inhibited (p>0.05). FACS analysis at day 14 showed that lenalidomide samples had higher proportions of cells expressing erythroid markers and lower proportions expressing myeloid markers. Gene expression profiling showed that four genes were up-regulated by lenalidomide in all MDS 5q− and control samples analyzed: ZIC4, SPARC, IL-6, and SPARC. The median up-regulation of SPARC was 4.3-fold (range 2.3−9.4). LRPIII and HB2A were down-regulated in 10 of 11 samples. Several of the differentially expressed genes warrant further investigation. The SPARC gene has been postulated to be a tumor suppressor gene in AML and has been shown to have anti-proliferative and anti-angiogenic effects. Interestingly, the SPARC gene maps within the commonly deleted region (CDR) of the 5q− syndrome at 5q31−q32. Conclusions: Lenalidomide selectively inhibits in vitro growth of 5q− hematopoietic progenitors, while not affecting growth of cytogenetically normal cells from MDS patients with 5q deletion or from healthy controls. In addition, lenalidomide affects cell differentiation and induces changes in gene expression including up-regulation of SPARC. We hypothesize that one part of the potent effects of lenalidomide is mediated through increased SPARC expression. Whether the localization of the SPARC gene to the CDR of the 5q− syndrome is significant or not to the pathogenesis of the 5q− syndrome remains to be determined.

**1032**

**CLINICAL BENEFIT FROM 2 PHASE II TRIALS EVALUATING LENALIDOMIDE (REVLIMID) IN LOWER-RIK MYELODYSPLASTIC SYNDROME PATIENTS WITH OR WITHOUT DEL 5Q CYTGENETIC ABNORMALITIES**

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Transfusion-dependent myelodysplastic syndrome (MDS) is a serious illness which adversely impacts overall survival. Results from 2 phase II clinical trials have shown that oral lenalidomide produces meaningful hematological improvement in patients with low- or intermediate-1 risk MDS with or without an associated del 5q cytogenetic abnormality (A. List et al EHA 2005, A. Raza et al. MDS 2005). To investigate possible differences in efficacy and safety of lenalidomide in lower-risk MDS patients with or without an associated del 5q cytogenetic abnormality. Results: From patients from 2 phase II clinical trials (MDS-002, MDS-005) of lenalidomide were analyzed. Differences in the frequency of red blood cell (RBC)-transfusion independence, improvement in hemoglobin, cytogenetic and pathologic response and safety were evaluated. In the del 5q patients, 67% (66/97) of the patients who had become RBC-transfusion independent remained RBC-transfusion independent. The duration of response was at least 24 weeks in 84% (83/97) of the responders and was at least 52 weeks in 53% (52/97) of the patients. The median increase in blood Hb from baseline to the maximum Hb achieved during RBC-transfusion independence was 5.5 g/dl (range, 1.1–11.4 g/dl, n = 99). Major cytogenetic responses were observed in 44% (32/72) and minor cytogenetic responses were observed in 29% (21/72) of the patients who were evaluable for cytogenetic responses. The patients with available follow-up bone marrow aspirate specimens, the follow-up bone marrow aspirates from 33% (27/81) of the patients had no evidence of MDS. In the non del 5q population, 26% (56/215) of the patients had achieved RBC-transfusion independence by ITT analysis during lenalidomide therapy. In this population, 77% had a normal karyotype and no differences were observed in transfusion independence rate between patients with a normal versus abnormal karyotype (29% and 27%, respectively). The duration of response was at least 24 weeks in 17% (36/215) of the responders and was at least 52 weeks in 10% (22/215) of the patients. Lenalidomide-induced transfusion independence was associated with a median increase from baseline in Hb of 3.0 g/dl in the responders. Neutropenia and thrombocytopenia were the most common adverse events and were reported at least once in 44% (172 and 174/395, respectively) of the patients who were treated with the 10 mg/day starting dose. Combined disease- and treatment-associated mortality (6%; 25/408) was relatively low and appeared consistent with other studies evaluating lenalidomide for MDS. LENALIDOMIDE is an effective and well-tolerated treatment for a select group of patients with low- or intermediate-1-risk MDS without an associated del 5q cytogenetic abnormality. From these data it becomes evident that there is a subpopulation of the non del 5q patients who respond similarly to del 5q patients and that these studies are warranted to investigate pathogenetic differences that account for the karyotype dependence in the frequency and durability of response.
VEGF and KDR. Inhibition of angiogenesis correlates with a significant reduction of the MDS clone in bone marrow whereas increase of vascularization indicates progression of disease.

1034
AN ABERRANT mRNA SPlicing PHENOTYPE IN MYELODYSPLASTIC SYNDROMES (MDS)
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Most mammalian genes undergo alternative pre-mRNA splicing, which generates diverse transcripts and protein isoforms that may be regulated in a tissue- or developmental stage-specific manner. Ablent and alternative splicing may be associated with neoplasia, and can contribute to alterations in gene expression detectable by oligonucleotide microarrays, though the function of the novel spliceforms in cancer pathogenesis is, for the most part, unknown. While studying ATRX, an X-linked gene encoding a chromatin-associated transcriptional regulator that was recently shown to be mutated in patients with MDS and an acquired thalassemic phenotype (Gibbons R et al. Nature Genetics 2003 and Steensma DP et al. Blood 2004), we discovered a novel exon-skipping and frameshifting alternative spliceform in the region of the gene that encodes the conserved helicase domain of the protein. A cis-acting genomic DNA mutation of ATRX was not detected, leading us to hypothesize that aberrant splicing as a consequence of trans-acting defects altering basal splicing machinery or regulators of alternative splicing might be common in MDS. To further characterize aberrant/alternative splicing of ATRX and other representative genes in MDS. We performed RT-PCR of marrow and blood cells from patients and healthy controls of ATRX, CDC25C (a gene at 5q31.1 that encodes a phosphatase important in cell cycle regulation, with expression that changes during lenalidomide therapy), and HELLS/SH/PASG/SMARCA6 (encodes a SWI/SNF2-related helicase that, like ATRX, localized to pericentromeric heterochromatin). Amplicons were analyzed by DNA sizing column chromatography under non-denaturing conditions, cloned into DH5α competent cells using the pGEM-T Easy system, and sequenced. The novel aberrant ATRX exon-skipping transcript was not present in 20 varieties of normal tissue from autopsies (gut epithelium, testis, myocardium, etc.), and was detected in blood cells from only 1 of 24 healthy volunteers (transiently). In contrast 7/13 patients with MDS and 4/16 patients with myeloid leukemia exhibited the ATRX variant in hematopoietic cells, in some patients in equal or greater proportion than the normal transcript. In MDS patients treated with chemotherapy who achieved a cytogenetic remission, the aberrant ATRX transcript disappeared, and was again detectable at the time of disease relapse. We also observed a series of novel exon-skipping or intron-retaining alternative spliceforms of CDC25C that were not present in healthy controls, and splicing patterns of HELLS/SH/PASG/SMARCA6 were likewise disrupted in MDS primary samples. A subset of patients with MDS may exhibit a generalized defect in pre-mRNA splicing that leads to the generation of aberrantly spliced isoforms in multiple genes of potential pathobiological relevance. The etiology and functional significance of these variants should be explored further. Because the nature of neoplasia-associated alternative gene products is often consistent with an active role in cancer, this suggests a potential therapeutic target in malignancies such as MDS that display aberrant splicing.

1035
AUTOLOGOUS GRAFT VERSUS HOST DISEASE (AUTO-GVHD) AFTER AN ALEMUTUMAB CONTAINING CONDITIONING REGIMEN AND AUTOLOGOUS STEM CELL TRANSPLANTATION IN CLL: IMMUNOLOGICAL MECHANISMS AND POTENTIAL ANTI-LEUKEMIA EFFECT
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A high incidence of unexplained skin rashes and auto-GvHD was observed after an alemtuzumab containing myeloablative conditioning regimen and autologous stem cell transplantation (SCT) in patients with CLL. 1) Comparison of CLL patients undergoing autologous stem cell transplantation after conditioning with TBI/Cy ± Alemtuzumab. 2) Detailed analysis of the defects of immune reconstitution. 3) Analysis of the influence of auto-GvHD on minimal residual disease. Methods. We retrospectively analyzed 26 patients with CLL (Binet 3C / 3B) with autologous SCT in two trials of the German CLL Study Group at the University of Ulm (CLL3 & CLL3C trials). Patients received cytoreduction with fludarabine plus cyclophosphamide and stem cell mobilization with Dexam-Beam. In the CLL3 trial (n=11) autologous SCT was performed after standard conditioning with 12 Gy total body irradiation and cyclophosphamide (120 mg/kg) (TBI/Cy). Patients in the CLL3C trial (n=16) were treated identically except for the addition of alemtuzumab before SCT (mean 100 mg iv) (Alem/TBI/Cy). There were no skin rashes or auto-GvHD in the standard TBI/Cy group. In contrast, 12 of 16 patients (75%) receiving Alem/TBI/Cy developed a skin rash (maculopapular rash (n=5), erythrodermia (n=3), eczema (3)) between 43 and 601 days after SCT. In 7 patients a clinical diagnosis of auto-GvHD was made. Typically, concurrent symptoms at the onset of auto-GvHD included conjunctivitis (n=4), sicca syndrome (n=5), and cholestasis (n=4). The histological findings were compatible with GVHD grade 1-2 in all five patients with clinical auto-GvHD in whom skin biopsies were performed. The median duration of GVHD was 517 days (range 60-867) and the reduction of immunosuppression led to a flare of the skin rash in 5 of 7 patients. The reconstitution of CD4 and CD8 positive cells was severely delayed in the Alem/TBI/Cy group with a particular depletion of CD8+ cells for up to 2 years. The CD4/CD8 ratio was abnormally high in the Alem/TBI/Cy group. This increased ratio was mainly caused by the extreme CD4/8 ratio imbalance in patients with GVHD. The CD4/8 ratio was 20 times higher among patients with auto-GvHD as compared to patients without GVHD (all time points combined). Interestingly, histology showed a predominant invasion of the skin by CD4 positive T lymphocytes. Molecular analysis revealed oligoclonal expansion of T cells in the skin biopsy samples of patients with GVHD with similar Vβ subfamilies (BV3, BV18) (n=3). The addition of alemtuzumab led to continuous MRD negativity in 10/14 patients (71%) compared to 0/7 patients receiving TBI/Cy (p=0.0039). Within the Alem/TBI/Cy group continuous MRD negativity was observed in 6/6 patients with auto-GvHD (100%) vs. 4/8 patients without auto-GvHD (50%; p=0.08). The current study demonstrates a remarkable incidence of a GVHD-like syndrome attributable to the addition of alemtuzumab to the TBI/Cy conditioning regimen before autologous SCT in patients with CLL. The addition of alemtuzumab to the conditioning regimen led to improved disease control at the molecular level and longer follow-up will show if the GVHD-like syndrome will lead to an anti-leukaemia effect and prolonged MRD negativity.
1036
PRELIMINARY RESULTS FOR THE PHASE III TRIAL OF ALETUZUMAB (CAMPATH) VS CHLORAMBUCIL AS FIRST-LINE TREATMENT FOR B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL)

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Chlorambucil (CHLO) is an approved therapy for patients with B-CLL. Alemtuzumab (CAM) has suggested effectiveness in untreated and demonstrated efficacy in relapsed and refractory B-CLL. CAM307 is an international, randomized, open-label study comparing efficacy and safety of CAM versus CHLO in previously untreated patients with B-CLL. Presented are the preliminary safety and efficacy results of this study. Eligible pts were Rai stage I-IV with evidence of progression requiring therapy. Patients with secondary malignancies, autoimmune thrombocytopenia, active infection, central nervous system involvement, or who were positive for cytomegalovirus (CMV) via quantitative PCR, were excluded from the trial. Patients were randomized 1:1 to receive either CAM 50 mg IV 5 times a week for up to 12 weeks or CHLO 40 mg/m² PO on day one of a 28-day cycle for up to 12 cycles. All patients in the CAM arm received prophylaxis with trimethoprim/sulfamethoxazole DS and falciclovid during therapy and until CD4 counts returned to ≥200 cells/µL. Accrual completed with 297 patients (213 males, 84 females; median age 60 yrs [range: 35-86]; CAM n=149 and CHLO n=148. Treatment arms were well balanced for key prognostic factors analyzed to date; overall, 96% were WHO PS 0-1, 70% had <3 cm lymphadenopathy. Median length of treatment was 11.7 wks for CAM and 24.4 wks for CHLO. An independent analysis of response showed an 82.6% Overall Response (OR) in the CAM arm compared to 54.7% OR in CHLO (p<0.0001), and patients in the CAM arm had a significantly higher CR rate compared to those in the CHLO arm: 22.1% vs 2.0% (p=0.0001). Preliminary analysis of the pertinent safety data reported through October 24, 2005 is summarized in the Table.

Table 1. Phase III CAM study.

<table>
<thead>
<tr>
<th>Safety</th>
<th>CAM (n=149)</th>
<th>CHLO (n=148)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombocytopenia</td>
<td>17.7%</td>
<td>15.6%</td>
<td>0.7546</td>
</tr>
<tr>
<td>Anemia</td>
<td>12.2%</td>
<td>15.0%</td>
<td>0.6103</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>42.2%</td>
<td>23.1%</td>
<td>0.0007</td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td>4.8%</td>
<td>3.4%</td>
<td>0.7697</td>
</tr>
<tr>
<td>Infection (excluding CMV)</td>
<td>14.3%</td>
<td>6.8%</td>
<td>0.0052</td>
</tr>
<tr>
<td>CMV infection</td>
<td>6.8%</td>
<td>0.0%</td>
<td>0.0017</td>
</tr>
</tbody>
</table>

*Comparisons were made using the Exact method.

Overall safety data indicate 34.7% of CAM patients and 19.7% of CHLO patients experienced a serious adverse event, with 21.1% and 4.1% considered drug related, respectively. One treatment related death occurred in the CHLO arm and none in the CAM arm. Infusion related events (fever, rigors, nausea, vomiting, and hypotension) were the most frequently reported CAM related events; however the severity was predominately grade 1/2 whereby only 13.6% of patients experienced a grade 3/4 event. The only pertinent grade 3/4 safety signals which attained statistical significance were neutropenia and CMV infection. As expected, the incidence of infection in the CAM arm was higher than the CHLO arm. However, there was no difference in the incidence of febrile neutropenia in the two study arms. Preliminary analysis of this randomized controlled trial shows that CAM had an OR and CR rate statistically superior to CHLO with manageable toxicity.

1037
CONSOLIDATION AND MAINTENANCE THERAPY WITH RITUXIMAB PROLONG DURATION OF RESPONSE BOTH WITHIN ZAP-70 positive and negative CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Clinical trials of monoclonal antibodies in combination with chemotherapy have reported improved outcome in CLL because this approach reduces disease burden to levels detectable only by flow cytometry or molecular methods. Along this line, we have recently published that rituximab in sequential combination with fludarabine (Flu) for symptomatic, untreated CLL allowed us to achieve higher remission rates and longer duration of response (Cancer, 2005). Recent literature data indicate that unmutated VH genes, CD38 and/or ZAP-70 protein tyrosine kinase overexpression may predict a worse outcome. We performed a phase II study that added rituximab sequentially to Flu as initial therapy for symptomatic, untreated CLL in order to evaluate both the clinical response and outcome. In about one third of the patients we added a consolidation/maintenance therapy with rituximab in order to prolong even more the response duration. ZAP-70 protein was determined before chemotherapy on mononuclear cells by flow cytometry using anti-ZAP-70 Alexa Fluor 488 (Caltag Laboratories) conjugated antibody. Seventy-two CLL patients, median age 60 years (range 37-74) received six monthly courses of Flu (25 mg/sqm for 5 days) and four weekly doses of rituximab (375 mg/sqm) starting on an average of thirty days (range 21-150) after completion of Flu therapy. According to modified Rai stages, 4 patients had a low stage, 67 an intermediate stage and 1 a high stage. Based on NCI criteria, 56/70 (80%) patients achieved a complete remission (CR), 12/70 (17%) a partial remission (PR) and 2/70 (3%) a stable disease (SD). Three patients presented grade 3 (WHO) infective lung toxicity and 1 patient acute fatal B hepatitis. Hematological toxicity included mainly neutropenia (grade 3 and/or 4 in 37 pts) and thrombocytopenia (grade 3 and/or 4 in 4 pts). Twenty six patients, either with CD5+CD19- bone marrow cells >1% (n=16 pts) or presenting CD19+CD5+ peripheral lymphocytes >1000/µL (n=10 pts) within six months after completion of the induction treatment, underwent consolidation/maintenance therapy with four monthly cycles of rituximab at 375 mg/sqm followed by eight/twelve monthly doses of rituximab at 150 mg/sqm. The median follow-up duration was 36 months. Noteworthy, all B-CLL pts experienced a very long progression-free survival (PFS) from treatment (71% at 5 years). Nevertheless, CLL patients that underwent consolidation therapy showed a significant longer duration of response (87% vs 54% at 5 years, p=0.02). ZAP-70 was positive (>20%) in 55/72 (49%) pts and a significant shorter PFS was observed in ZAP-70+ pts (36% vs 95% at 5 years, p=0.0002). Noteworthy, within the consolidated patients subset (n=26), ZAP-70+ pts (n=11) showed a worse PFS (67% vs 100% at 5 years, p=0.02). However interestingly, within the ZAP-70- subset (n=35), the consolidated patients (n=11) showed a significant longer duration of response (67% vs 0% at 2.5 years, p=0.02, Figure) in comparison with non consolidated patients (n=24). Therefore, the addition of consolidation/maintenance therapy with rituximab significantly prolongs duration of response allowing a better outcome. Finally, this immunotherapeutic supplement seems to improve significantly the clinical outcome of pts notoriously with a bad prognosis, such as ZAP-70+B-CLL.
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LUMILIXIMAB IN COMBINATION WITH FLUDARABINE, CYCLOPHOSPHAMIDE, AND RITUXIMAB (FCR) FOR PATIENTS WITH RELAPSED CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Introduction: Lumiliximab (OBI-002), an IgG3 monoclonal antibody, has B-cell specificity and has been shown to induce apoptosis in B-cells. Preliminary data showed that lumiliximab has activity in patients with relapsed chronic lymphocytic leukemia (CLL). A phase 1, open-label, dose-escalation study was conducted to determine the safety profile of lumiliximab in combination with fludarabine (FD) and cyclophosphamide (C). The objectives of this study were to determine the safety and efficacy of lumiliximab in combination with fludarabine and rituximab (FCR) in previously treated patients with CLL.

Methods: Patients (n=31) with relapsed or refractory CLL were eligible for the study. Patients received lumiliximab in combination with fludarabine and cyclophosphamide every 2 weeks (range, 1 to 9), median age of 57, 60% were males, 95% had WHO performance status of 1. Fourteen patients experienced CTC Grade 3 or 4 adverse events. Events reported in more than 1 patient were neutropenia (7 patients), leukopenia (4 patients), febrile neutropenia (3 patients), thrombocytopenia (2 patients), and pyrexia (2 patients). Hematologic toxicity is comparable with the FCR regimen. Sixteen patients completed ≥3 cycles of treatment and 4 patients completed ≥2 cycles. Response was evaluated using NCI-WG criteria. Nine (45%) patients have confirmed complete responses, 5 (25%) have partial responses, and 6 (30%) patients have disease progression.

Conclusions: Lumiliximab in combination with FCR is well tolerated and has shown no increased toxicity compared with FCR alone. Lumiliximab may enhance the CR rate with FCR when used for treatment of patients with progressive CLL after prior therapy.

1039

INTERIM REPORT OF THE UKCLL02 TRIAL: A PHASE II STUDY OF SUBCUTANEOUS ALEMTUZUMAB PLUS FLUDARABINE IN PATIENTS WITH FLUDARABINE REFRACTORY CLL (ON BEHALF OF THE NCRI CLL TRIALS SUB-GROUP)

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Patients with fludarabine refractory CLL have a median survival of 10 months with conventional chemotherapy. Intravenous (IV) alemtuzumab is approved in fludarabine refractory CLL resulting in 35 to 50% responses. Combined alemtuzumab and fludarabine can induce responses in CLL refractory to both agents. Infusion reactions and 2-hour infusions 3x a week for 12 weeks are problems with IV alemtuzumab. Subcutaneous (SC) alemtuzumab is more convenient but pharmacokinetics suggest the need for prolonged therapy with little efficacy data in fludarabine-refractory CLL. A study to assess the safety and effectiveness of SC alemtuzumab in fludarabine-refractory CLL. Methods: A study to assess the safety and effectiveness of SC alemtuzumab in fludarabine-refractory CLL. Methods: SC alemtuzumab was given at a dose of 30 mg 3x a week (after dose escalation) for up to 24 weeks depending on 6-weekly marrow assessments. Patients failing to respond to alemtuzumab in the trial could receive oral fludarabine (40 mg/m2/day for 3 days every 4 weeks) combined with SC alemtuzumab. In this planned interim analysis of the first 44 patients (median age 66, range 41 to 79) 2 patients died before receiving alemtuzumab, and 5 remain on therapy. Of the remaining 37 patients, one withdrew consent and 36 patients have completed therapy. Responses to alemtuzumab monotherapy (n=36) were 2 MRD negative CR, 1 MRD positive CR, 11 PR (including 1 MRD negative patient who remained cytopenic), 20 NR and 2 died. Alemtuzumab was given for a median 12 weeks (range: 2-24) with a median dose of 913 mg (range 106 to 2173 mg). 12 patients (8 NR and 4 PR) received concurrent fludarabine and SC alemtuzumab (median 2.5 courses fludarabine [range 1-3]). Two non-responders achieved a PR and one of the partial responders achieved a CR (MRD positive). Therefore the overall response rate for the whole cohort was 16/36 (44%) including 3 MRD negative patients (2 CRs and 1 PR). IgVH gene was unmutated (<98% homology to germ line DNA) in 11/14. FSH revealed poor risk deletions (11q and/or 17p) in 21/34 patients (17p- in 9; 11q- in 6 and both in 6). p53 functional analysis is available for 23. 20/23 had p53/ATM dysfunction or deletion. 13/25 (52%) of patients with poor risk deletions (11q and/or 17p) or p53 dysfunction responded to therapy. The initial alemtuzumab dose was associated with localised erythematous skin reactions in 20 patients (diameter 1 to 18 cm), fever in 7 and rigors in 3. All reactions subsided in <48h. Serious infections during alemtuzumab monotherapy were: CMV reactivation (10); febrile neutropenia (9); invasive fungal infection (8); pneumonia (2). On the combination, CMV reactivation in 2 cases but no other grade 3+ infections. All CMV reactivations resolved on antiviral therapy. Grade 3+ thrombocytopenia and neutropenia was seen in 16 and 25 patients on alemtuzumab monotherapy as well as in 1 and 2 patients on combined therapy, respectively. We report that subcutaneous alemtuzumab is effective in poor-risk fludarabine-refractory CLL and is well tolerated compared to IV therapy. A longer duration of SC alemtuzumab therapy (up to 24 weeks) is required. The addition of oral fludarabine improves the response rates with acceptable toxicity.
**Clinical studies in Non-Hodgkin's Lymphoma**

**1040** SURVIVAL IMPACT OF THE TIME REPEATED BCL2/Igh REARRANGEMENT MEASUREMENTS IN FOLLICULAR LYMPHOMA PATIENTS TREATED WITH FRONT-LINE AUTO-TRANSPLANTATION IN THE GELF-94 TRIAL BY THE GELA

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**Backgrounds.** This study was undertaken to assess the impact of molecular residual disease (MRD) on survival controlling for consolidation treatment of two regimens: an autotransplantation framework or chemotherapy with interferon, in the treatment of high burden follicular lymphoma. MRD was defined as disappearance of Bcl2-IgH amplification by PCR. Aims. From 07/94 to 03/01, we have performed a prospective study, the GELF-94 trial, which randomized consolidation treatment after achieving clinical response between front line ASCT and chemotherapy. Of 401 patients included, 209 received 12 cycles of CHPV (cyclophosphamide, doxorubicin, vincristine and prednisone) plus interferon α (18 months (CHPV-I arm) and 192 received 4 cycles of CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) then high-dose therapy with total body irradiation and ASCT (CHOP-HDT arm)). Methods. Bone marrow (BM) and peripheral blood (PB) samples were obtained prospectively at diagnosis and repeated every 6 months during the first year then annually for PCR analysis in 12 laboratories. A standard PCR technique with one step of amplification was used for MBR and mcr breakpoints. Time repeated measurement was stopped at clinical relapse or instigation of a new treatment. At diagnosis, 225 patients had material available for Bcl2/Igh rearrangement analysis: BM for 182 patients (3%) and no rearrangement at MBR or mcr in the remaining 94 patients (44%). No differences were found according to Bcl2/Igh rearrangement in terms of response rate (82% for bcl2- vs. 80% for bcl2+) and 5 years survival (85% for bcl2- and 79% for bcl2+). Time repeated Bcl2/Igh rearrangement measurements were available for 142 patients (ASCT n=75, chemo n=67); in BM for 79 patients and in blood for 85 patients. There was no statistically significant difference in clinical characteristics between patients with/without time repeated measurement. Results. At a median follow-up of 64 months, the significant prognostic factors for survival were age below 40 years (RR=21, p=0.005), complete clinical response (RR=5, p=0.02) and bcl2/Igh rearrangement negativity (RR=5, p=0.08), by time dependent Cox’s model. There was no treatment impact. This findings confirm the importance of molecular response in addition to the clinical response as a critical factor for prognosis. Conclusion. No matter whether after chemotherapy alone or after autologous bone marrow transplant, patients in complete clinical and molecular remission show a significantly longer overall survival.

**1042** GAINS ON CHROMOSOME BAND 18Q21 PREDICT POOR OUTCOME IN PATIENTS WITH DIFFUSE LARGE-B-CELL LYMPHOMA: RESULTS FROM A COMPARATIVE GENOMIC HYBRIDIZATION ANALYSIS WITHIN A MULTICENTRIC TRIAL (NHL-B-TRIAL)

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**Backgrounds.** In Non-Hodgkin lymphomas, there are only few data regarding the prognostic significance of specific genomic aberrations. No analyses correlating genomic aberrations with the clinical course have been performed in homogenous cohorts of patients treated within a clinical trial. Aims/Methods. We used comparative genomic hybridization (CGH) to perform such an analysis in diffuse large B-cell lymphomas (DLBCL). 367 paraffin-embedded tumor samples were obtained from patients, who where treated within the NHL-B trial of the German High-Grade Non-Hodgkin’s Lymphoma Study Group. In this trial, all patients received similar therapy regimens (CHOP or CHOP-E adminis-tered every 14 or 21 days). Results. CGH analysis was successful in 256 out of 367 cases (70%). 186 patients out of this series had a histology of DLBCL according to the WHO classification. In 137 of 186 cases (74%), imbalanced chromosomal aberrations were found (range from 1 to 25; median 4.5). The most frequent chromosomal changes (>15% of all cases) were gains involving chromosome bands 1q21 (16%), 7q21 (15%), 7q11 (16%), 17q22 (16%), 8q24 (16%), 11q22 (22%), 6q21 (23%) and 14q21 (17%). 43 high-level DNA amplifications were found in 26 cases, most frequently involving the chromosomal bands...
18q21 (14 cases) and 2p13 (5 cases). Median follow-up time was 67 months (range 0.4-140). In the univariate analysis, gains on chromosome 3 (3p14: p<0.001, overall survival (OS), p=0.001, time to treatment failure (TTF); 3q22: p<0.001, OS, p=0.002, TTF; 3q27: p<0.001, OS, p=0.001, TTF), on chromosome arm 12p12 (p=0.05, OS, p=0.06, TTF) and on chromosome arm 18q21 (p=0.001, OS, p=0.002, TTF), as well as losses on 17p13 (p=0.09, OS, p=0.001, TTF) were associated with an inferior prognosis. In a multivariate model including the clinical parameters of the International Prognostic Index (IPI), 18q gain remained as an independent negative prognostic marker (OS: relative risk: 2.1 (1.3-3.5), p=0.004; TTF: relative risk: 1.9 (1.2-3.0), p=0.006). Subset analysis revealed that 18q gains are particularly predictive in the IPI-low and intermediate-low group (p<0.01). In 130 cases, molecular cytogenetic studies were bcl-2 expression were performed. The negative prognostic impact of bcl-2 expression for overall survival was restricted to cases with an additional 18q gain (p=0.02). **Summary:** These data demonstrate that molecular cytogenetic studies can be performed in the context of a clinical lymphoma trial. Genomic changes can improve the risk assessment in DLBCL.

### 1043 ASSESSMENT OF DISEASE DISSEMINATION IN GASTRIC VERSUS EXTRAGASTRIC MALT LYMPHOMA USING EXTENSIVE STAGING: A SINGLE CENTER EXPERIENCE OF 140 PATIENTS

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**Backgrounds.** Molecular data as well as preliminary clinical findings have suggested MALT lymphoma as a multifocal disease in a high percentage of patients. We report our findings with an extensive staging routine applied in patients diagnosed with MALT lymphoma at our institution. **Patients and Methods.** A total of 140 consecutive patients underwent staging according to a standardized protocol. Sixty-one had gastric lymphoma, while 79 had been diagnosed with extragastric MALT lymphoma. The majority of these latter patients had salivary gland lymphoma (n=24, 30%, with 22 parotid and 2 submandibular gland lymphomas), 17 had lymphoma of the orbit/lacrimal gland (20%), another 11 had MALT lymphoma originating in the lung (14%) and 10 had primary intestinal MALT lymphoma (12.5%, 8 in the colorectum and two in the small intestine). The remaining patients suffered from lymphoma of the thyroid (n=5), conjunctiva (n=4), the breast (n=5), the liver (n=2) and the kidney (n=2). Staging included gastroscopy with multiple biopsies, endosonography of the upper gastrointestinal (GI) tract, computed tomography (CT) of thorax and abdomen, lymph node sonography, ophthalmoscopy with multiple biopsies, otorhinolaryngologic assessment, MRI of salivary and lacrimal glands, and bone marrow biopsy. All lesions suggestive of lymphoma involvement were subjected to biopsy, if accessible, and biopsies were evaluated for MALT-lymphoma specific genetic aberrations by means of RT-PCR and/or FISH. These include gains on chromosomes 3 and 18 was significantly higher in patients with extragastric MALT lymphomas (p<0.004 for trisomy 3 and p=0.037 for trisomy 18), as was t(14;18) involving IGH/MALT1 in 22% of extragastric MALT lymphomas (p=0.045) and with trisomy 18 in extragastric lymphomas (p=0.011). **Conclusions.** Our findings suggest that MALT lymphoma frequently presents as a multifocal disease. Extragastric MALT-lymphomas are significantly more prone to dissemination than gastric MALT lymphomas.
The association between gastrointestinal angiodysplasia and von Willebrand’s disease (vWD) is uncommon. Since the first description in 1967, some other 20 cases have been reported; most of them were vWD type 2 and 3. The efficacy of several therapies has been inconsistent and transient: transfusions, factor VIII / vWD concentrates, endoscopic sclerosis, estrogens, surgery. Bowers et al. published 2 cases with this association in whom a significant decrease in transfusion requirements and episodes of hemorrhage was obtained through the use of octreotide (Br J Haematol. 2000 Mar;100(3):324-7). The therapeutic effect of this synthetic analogue of somatostatin in this setting lies on the reduction of splanchic blood flow to abnormal blood vessels. In one of their patients, an unforeseen increase in vW factor activity was also demonstrated. We describe an additional case with protracted use of octreotide in a long-acting formulation. 61 yr. old, female, allergic to iodine (anaphylaxis). vWD type 2b. Chronic hepatitis C of presumed post-transfusional origin, genotype 1. Multifocal angiodysplasia in gastrointestinal tract with recurrent episodes of upper and lower bleeding (>100 days of hospital stay and >100 blood products per year). Previous therapies: surgery (partial gastrectomy; hemicolectomy); combined estrogens and progesterone. She refused prophylactic use of vWF / factor VIII derivatives.

### Table 1. Evolution of parameter.

<table>
<thead>
<tr>
<th>Years products</th>
<th>Blood to hospital</th>
<th>Admittances of stay</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>137</td>
<td>18</td>
<td>123</td>
</tr>
<tr>
<td>2000</td>
<td>168</td>
<td>22</td>
<td>167</td>
</tr>
<tr>
<td>2001</td>
<td>87</td>
<td>16</td>
<td>106</td>
</tr>
<tr>
<td>2002</td>
<td>131</td>
<td>16</td>
<td>122</td>
</tr>
<tr>
<td>2003</td>
<td>80</td>
<td>13</td>
<td>96</td>
</tr>
<tr>
<td>2004</td>
<td>77</td>
<td>9</td>
<td>90</td>
</tr>
</tbody>
</table>

In the second semester of 2000, we obtained permission for compassionate use of octreotide; the initial therapeutic scheme included a progressively higher dose to reach 250 µg SC t.i.d., even though compliance to side effects prevented a dose higher than 250 µg SC b.i.d. At the end of 2000, the conventional formulation was replaced by long-acting-release (LAR) octreotide, 20 mg IM monthly, each dose preceded by 1000 IU of a factor VIII / vWF concentrate to minimize local bleeding; both octreotide and factor were administered in the Day-In Hospital. The favourable evolution since then, with regard to decrease in number of hospital admittances, days of hospital stay and number of blood products transfused, is depicted on Table 1. In 2002, there was an apparent loss of response, related to a single episode of hemoperitoneum. In 2004, two of the admittances were due to thrombosis and infection of the central venous catheter, which required heparin therapy. When comparing data from 1999 and 2000 on one side, and those from the following 4 years on the other, the number of transfused blood products, hospital admittances as well as days of hospital stay, diminished by a gross one third (39%, 35% and 30%, respectively), with a trend to further decrease. In addition, this has led to a significant improvement in the quality of life for the patient, allowing her a ‘life beyond the Hospital’. The toxicity has been scarce, with a slight increase in blood pressure and glucose levels as the only relevant events. We believe that octreotide-LAR is an option to be considered in those patients with recurrent bleeding related to vWD and gastrointestinal angiodysplasia.

**References**

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Dyskeratosis congenita (DC) is rare, usually fatal inherited syndrome. LDL-apheresis is a method of extracorporeal elimination of serum LDL-cholesterol that is used for treatment of patients with severe hyperlipidemia resistant to diet and pharmacotherapy. Applicable markers that could be used to determine efficacy of this treatment to lower the activity of atherosclerosis are still to be found and remain unresolved. Activity of primary haemostasis plays an important role in the development of atherosclerotic complications. Aims. We hypothesize that investigation of primary haemostatic activity could be a quick and useful marker for monitoring LDL-apheresis efficacy. The aim of this study was to verify this hypothesis. Methods. Commercial analyser Dade Behring PFA-100, Germany (PFA, platelet function analyse) was used.
for all investigations. This analyzer enables quantitative measurement of platelet-mediated haemostasis in noncoagulable (citrated) blood. The method simulates platelet activation by mechanical stress - shear stress, and also simulates contact of platelets with collagen. There were 9 patients with familiar hypercholesterolemia in the study group (4 females and 5 males). Age ranged from 17 to 59 years (46.4 years average and 35 years median), 2 of them have homozygous hypercholesterolemia. Our aim was to investigate the changes before and after procedure two times in every patient. Results. 18 pairs of samples were examined using COL/EPI membrane (collagen/epinephrine) and 17 pairs of samples were examined using COL/ADP membrane (collagen/ADP), total number of samples was 70. Closure time (CT) values were prolonged after separation in all cases but CT prolongation was not statistically significant (p=0.14). No differences between homozygous and heterozygous patients were found. Summary/Conclusions. Investigation of primary haemostasis immediately after procedures using FFA-100 analyser is not a suitable marker and could not be used to determine the optimal intensity of particular LDL-apheresis procedures.

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1052 TWINNING IS A SIGNIFICANT INDEPENDENT FACTOR IN THE DEVELOPMENT OF CHILDHOOD MALIGNANCIES

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In the past few years we have noticed a marked increase in the incidence of cancer among twins treated at the Pediatric Hematology-Oncology Unit (PHOU) of Soroka Medical Center (SMC) in Beer Sheva, Israel. In this work, we reviewed the relationship between twinning and the risk of developing cancer during early childhood. Children born at SMC between Jan 1991 and Sep 2008, with any malignancy were included in the study if they were under 13 years-of-age at time of diagnosis. Three controls of the same sex were matched to each patient from the birth registries of the same day at SMC. A twin was not chosen as a control to its sibling. Data from patients, controls, and mothers were collected from medical records and included three areas of investigation: demographics and obstetric history of the mothers, delivery data, and gestational events and/or interventions (infertility including in vitro fertilization (IVF), ART, diabetes mellitus (DM), hypertension (HTN), urinary tract infections (UTI), iron deficiency anemia (IDA), and medications). A total of 145,087 deliveries, resulting in 145,503 children, were registered at the Soroka Medical Center (SMC) between January 1991 and September 2008. Of those, 98.37% were singleton and 1.63% were multiple births (2,261 twins; 77 triplet and quadruplets), cumulating in 972,000 patient years of follow up. The crude incidence of cancer during childhood is 14:100,000 per year, while the incidence of cancer calculated for the children born during the study period was 10.5:100,000 per year. Of the 92 children with cancer, complete information was obtained for 65 patients (70.6%); eight (12.3%) were twins, four were born after ART (6.7%), two of whom were twins. Significantly more Bedouin children were found in the patients group. According to this data, the total expected number of cancer cases among twins born during the study period was 2.23, while the total observed number at the PHOU is 5.58 times higher (β cancer cases), p<0.001. Twinning per se was found to be an independent factor in the development of childhood cancer. Although seeming significant in a univariate analysis, this increase may be a marker of total body iron storage in patients with thalassemia. Material and Methods. 84 healthy children as control group were compared with 71 thalassemia major, 10 thalassemia intermedia and 15 thalassemia trait. Salivary and serum iron and ferritin levels were measured in all groups. Results. there was no statistically significant difference between the control group and other groups by means of age and gender (p>0.05). There was no correlation between serum and salivary iron and ferritin levels in thalassemia major, intermedia and trait groups (Table).

Table. Correlation between serum and salivary iron and ferritin levels in patients thalassemia.

<table>
<thead>
<tr>
<th>Salivary and Serum Iron</th>
<th>Salivary and Serum Ferritin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>r=0.885, p=0.000*</td>
</tr>
<tr>
<td>T. Major</td>
<td>r=0.972, p=0.000*</td>
</tr>
<tr>
<td>T. Intermedia</td>
<td>r=0.891, p=0.001**</td>
</tr>
<tr>
<td>T. Trait</td>
<td>r=0.955, p=0.000**</td>
</tr>
</tbody>
</table>

As a conclusion, salivary iron and ferritin levels increases as well as serum iron. This increase may be a marker of total body iron storage in patients with thalassemia. Therefore non invasive, salivary samples for measurement of iron and ferritin may prefer instead of blood samples in patients with thalassemia. For this reason, we think that more extensive and controlled studies are needed to use the saliva as a routine diagnostic material.

1054 FERTILITY AND REPRODUCTION IN THALASSEMIA MAJOR

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Backgrounds. Therapeutic advances in thalassemia major have increased the average lifespan and improved the quality of life patients. Attainment of reproductive capacity and creation of a family has become a challenging task. Hypogonadotrophic hypogonadism due to hemosiderosis is still present and become a barrier in their desire for parenthood. Nowadays women with thalassemia can safely complete pregnancy, but the decision to conceive has to be carefully considered by a couple in consultation with their doctors. Patients with thalassemia who have a normal menstrual cycle may conceive spontaneously. Those suffering from primary or secondary amenorrhoea can be submitted with hormonal treatment in order to stimulate the production of ova and the induction of ovulation. Aims. Aim of our study is to estimate the frequency of fertility (spontaneous or after induced ovulation) and pregnancy complications for mother and newborn. In patients admitted to the Paediatric Department- Thalassemia Unit at Sacred Heart Hospital Ward G. Martino Policlinico, Palermo.

Methods. We followed 36 women mean age 32 (18-46) years. All patients were treated according to the standard treatment protocol. 9/36 women with Thalassemia Major became pregnant and were the object of our study. At the beginning of pregnancy, average age was 26 years. Five pregnancies were spontaneous and four were induced after ovarian stimulation followed by natural insemination. Women who expressed the desire to become pregnant underwent a complete evaluation of psychological and clinical conditions. Glucose tolerance, thyroid, serum ferritin levels, hepatic and renal function tests, bidimensional echocardiography were performed before, during the pregnancy and after delivery. Also Bone Mineral Density (BMD) was measured, by the DEXA method, before pregnancy and after delivery. Once the patients were confirmed to be pregnant, iron chelator treatment was stopped. Mean pre-transfusional Hb was 10-10.5 g/dl. 36 pregnancies were performed every two weeks. Results. Our findings show that 8 babies were delivered by elective caesarean section at 37° weeks of gestational age. The mean birthweight of the newborns was 2954 g. All babies were normal. Ferritin levels increased during pregnancy in all
patients. After delivery all of them were in good general conditions and were treated with intensive iron chelation in order to reduce iron overload. No changes in laboratory parameters, BMD, ecocardiography evaluation hepatic and renal functions, have been observed besides increased iron stores. There were no delivery complications but one case of intrauterine death at the 35th weeks due to acute placental injury and one abortion in the early pregnancy were reported. Conclusions. Pregnancy can be safe in mothers and babies if closely monitored. Reproduction in patients with thalassaemia major is becoming a new reality, allowing them an improved quality of life. Maternity desire has to be considered with special caution and sensitivity. An optimal relationship has to be reached between patients and care-givers to improve patients’ safety. This demands a complex effort and embraces all disciplines and sectors requiring a comprehensive, multifaceted approach to identify and manage actual and potential risks.

**1055 EFFICACY AND SAFETY OF INTRAVENOUSLY ADMINISTERED IRON SUROSCE (VENOFER) IN IRON DEFICIENCY ANEMIA PATIENTS**

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Conventional oral iron administration in iron deficiency anaemia (IDA) patients is simple, effective and tolerable. Nevertheless, there are patient cohorts in which an alternative method of iron delivery is required. These groups include patients with inflammatory bowel disease related malabsorption and bleeding, end stage renal patients receiving erythropoietin, patients with unresolved ongoing bleeding requiring in excess of the acceptable oral dose and patients exhibiting severe gastrointestinal adverse effects to oral iron. Intravenous iron administration provides a simple, practical alternative for these patients allowing the delivery of far greater doses than the oral route. Reluctance in prescribing this treatment results from previously reported serious adverse effects of iron dextran. We present retrospective data from 53 episodes of IDA patients (N=47) treated with intravenous iron sucrose (Venofer). The objective of this retrospective study was to determine the efficacy and safety of Venofer in IDA patients unable to tolerate oral iron administration. A total of 47 IDA patients (Number of episodes (N)=55; F=41; M=12) whom were clinically assessed to require an alternative route of iron administration received appropriate doses of Venofer (I=2000 mgs; 1=1600 mg; 1000 mgs; 5=800 mgs; 4=400 mgs, mean dose=964 mgs). Mean ages were 57 and 60 years for males and females respectively.

**Table 1. Mean differences and T-test p-values.**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mean improvement</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dL)</td>
<td>FERR (µg/L)</td>
<td>MCV (FL)</td>
</tr>
<tr>
<td>ALL patients</td>
<td>2.0 (p&lt;0.001)</td>
<td>113.5 (p=0.001)</td>
</tr>
<tr>
<td>ACD</td>
<td>1.12 (p=0.047)</td>
<td>128.8 (p=0.011)</td>
</tr>
<tr>
<td>CKD</td>
<td>2.58 (p=0.008)</td>
<td>154.8 (p=0.008)</td>
</tr>
<tr>
<td>GAST</td>
<td>2.01 (p=0.001)</td>
<td>69.5 (p=0.001)</td>
</tr>
<tr>
<td>Menor</td>
<td>3.0 (p=0.002)</td>
<td>118.3 (p=0.022)</td>
</tr>
</tbody>
</table>

Patients were anaemic due to either anemia of chronic disease (ACD) (F=9; M=0), chronic kidney disease (CKD) (F=5; M=3), gastrointestinal related diagnosis (GAST) (F=22; M=9) or menorrhagia (MENOR) (N=7). Haemoglobin (Hb), mean cell volume (MCV), mean cell hemoglobin (MCH) and ferritin (FERR) parameters were determined pre- and post- Venofer infusion and results analysed statistically to determine mean improvement, normality and significance. Laboratory results for the whole data set (table 1) were analysed retrospectively using T-tests of mean difference. Results show an overall statistically significant improvement in Hb (2.0g/dL), FERR (115.3±5.9/L), MCV (6.9FL) and MCH (2.8pg). Results categorised according to IDA precipitating condition show significant differences between Hb and FERR measured pre- and post- Venofer administration in all cohorts. The greatest improvement in Hb and FERR were seen in the MENOR and CKD patient groups respectively. Venofer was well tolerated, one patient was excluded after the first dose due to onset of nausea, vomiting, tachycardia and a slight drop in systolic blood pressure. Intravenous Venofer administration is a well tolerated, effective alternative to oral iron in IDA patients, particularly in IDA secondary to menorrhagia and CKD. The mean Hb difference rises by 0.003g/dL and mean FERR by 0.09 µL for every 1mg increase in Venofer dose. Implications for the patient and the clinician include training on self administration for safe, effective home therapy.

**1056 POST-TRAUMATIC STRESS DISORDER IN CHILDREN AFFECTED BY SICKLE CELL DISEASE AND THEIR PARENTS**

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**Backgrounds.** Children affected by SCD suffer from recurrent painful crises, some of them being life threatening, or felt as life threatening by children and/or their parents. The aims were to hypothesised that painful crises could generate PTSD in some children, PTSD being described in patients having experienced or witnessed an event that involved an actual or threatened injury to physical integrity of self or others. Patients with PTSD present symptoms in each of the categories: re-experiencing, avoidance/numbing, increased arousal. Until now, PTSD had never been described in SCD children. Methods. We enrolled 11 SCD children, 9 males, 2 females hospitalized at least once for a painful crisis, at least one month before the study, who accepted with one of their parents to participate in the study. Their mean age was 11.6±2.2 years (range: 7.5-15.5). One father and 10 mothers were also studied, one mother being secondarily excluded from analysis because she did not answer the questions. Both children and parents had to answer to a semi-structured interview (SCID) and to complete questionnaires (IES-R, STAY-Y, BDII, CDI, CBCL). Socio-demographic data and medical past histories were recorded. Statistical analysis used exact Fischer test and Mann Whitney test. Results. Three children had a PTSD (27%), and 4 parents (40%). We found no correlation between PTSD and socio-demographic data. Mean numbers of hospitalization/year were respectively 1.1±0.5 and 1.3±0.6 and mean number hospitalization in intensive care units respectively 1.3±0.6 and 1.5±0.8 in children with and without PTSD (N.S.). PTSD presence was not correlated in children and parents. There was a correlation between PTSD and the parents’ feeling of powerlessness on their child’s illness (p=0.04). Summary/Conclusions. Painful crises are the most frequent complications of SCD, and one of the most difficult question for their management is the assessment of pain intensity. We show here that PTSD could be a complication of SCD. Symptoms such as intrusive distressing recollections of past painful events may worsen the consequences of vasooclusion, enhancing children’s feeling of pain, and leading physicians to an inadequate use of analgesics instead of psychological support. Furthermore, stress is a precipitating factor for vasoocclusive crises and may facilitate recurrences of painful episodes. Moreover, PTSD itself causes psychological distress and may disturb children’s development. Interestingly, PTSD was not related in our study to the objective severity of the disease. Looking for PTSD and proposing specific individual and family psychological interventions could very probably contribute to disrupt the vicious circle between pain and fear of pain in SCD children.

**1057 TRANSFUSIONAL REQUIREMENTS ARE A KEY FACTOR IN TAILORING THE OPTIMAL DOSE OF CHELATION THERAPY, AS DEMONSTRATED BY THE NOVEL, ORAL IRON CHELATOR DEFEASIRASOX (EXJADE, ICJ670)**

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**Backgrounds.** The novel, once-daily oral iron chelator defesirasox (Exjade, ICJ670) has recently been approved in eight countries, including the USA and Switzerland, for the treatment of chronic transfusional iron overload in patients aged ≥2 years. Data from defesirasox clinical trials demonstrated a clear dose response, allowing physicians to tailor the dose to meet a patient’s therapeutic goal, ie maintenance or reduction of body iron burden stratified according to the transfusional requirements. The objective of this post-hoc, cross-study analysis was to evaluate the change in body iron burden stratified according to the transfusional requirements of iron overloaded patients during treatment with defesirasox or deferoxamine (DFO, Desferal®). Methods. Body iron burden was evaluated by pooling liver iron concentration (LIC) and serum ferritin data from four pivotal defesirasox clinical trials of up to 1 year’s duration. In total, 1,005 patients with a variety of transfusion-dependent anaemias were enrolled: 652 were randomized and treated with DFO. All patients were stratified into three categories depending on their transfusional requirements while on study: <7 (low), 7-14 (intermediate) or >14 (high) mL/kg/month of
packed red blood cells; 7 and 14 mL/kg/month correspond with approximately 2 and 4 adult units of blood, respectively. Results: In the overall population, 146 (22.4%), 419 (64.3%) and 87 (13.3%) patients who received deferasirox while on study had low, intermediate and high transfusional requirements, respectively; the equivalent numbers for patients receiving DFO were 61 (17.3%), 255 (66.6%) and 57 (16.1%), respectively. In completing patients with an LIC assessment at baseline and study end (approximately 90% of the overall population), a transfusion- and dose-related pattern was observed in the response of LIC (Figure 1) and serum ferritin. This was observed for both deferasirox (n=566) and DFO (n=327). Changes in iron burden were similar at comparable therapeutic doses. Conclusions: Transfusional requirement has a clear impact on response to chelation therapy. Physicians are able to tailor deferasirox dose according to patient needs, with dosing based on transfusion rate, severity of iron overload and treatment goal. Using this method, deferasirox was shown to meet the individual requirements of an extremely high proportion of patients treated. Deferasirox 10 mg/kg/day maintained iron balance in patients with low transfusional requirements, 20 mg/kg/day maintained or reduced iron balance in patients with low and intermediate requirements, while 30 mg/kg/day reduced iron balance in patients with high transfusional requirements. As regular transfusions lead to rapid iron accumulation, it is essential to monitor patients for the number of blood units transfused, serum ferritin and LIC. Across a range of transfusion-dependent anematic and transfusional requirements, DFO and deferasirox in a 2:1 dose ratio have comparable effects on LIC and serum ferritin.

Figure 1. Change in LIC (mg Fe/g dw), by treatment, dose and transfusional requirements.*

*Data not shown for the deferasirox 5 and DFO <25 mg/kg/day dose cohorts due to low patient numbers in these cohorts.

1058
IRON OVERLOAD CAUSED BY REPEATED TRANSFUSIONS IN ADULT CHRONIC REFRACTORY ANEMIAS
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Many patients with refractory anemia suffer from iron overload and associated complications. However, it is not yet established when to start deferoxamine therapy to prevent this. To analyze the clinical features of iron overload caused by repeated transfusions in adult chronic refractory anemia, we chose patients who received more than ten units of RBC cells from the database of blood bank in our institute and their medical records were retrospectively reviewed. Twenty-nine patients (M: 18, F: 11) were identified and median age was 52 years (range: 22-82). Underlying disease causing repeated transfusions was aplastic anemia in 11 patients, myelodysplastic syndrome in 9, acute myeloblastic leukemia in 4 patients, myelodysplastic syndrome in 3, and paroxysmal nocturnal hemoglobinuria in 1 patient. Each of the remaining patients had pure red cell aplasia, chronic lymphocytes leukemia, non-Hodgkin’s lymphoma, chronic myelogenous leukemia and acute myelogenous leukemia. Patients received median 85 units of RBC transfusions (range: 26-226). Two patients received 45, 101 units of RBC transfusions and developed liver cirrhosis after 143, 148 months from the initial diagnosis of their underlying diseases, respectively. Cardiomyopathy developed in 4 patients after 29, 100, 104, and 143 months from the diagnosis of underlying diseases. They received 71, 103, 115 and 133 units of RBC, respectively. Diabetes mellitus developed in 5 patients. Twenty-four patients started deferoxamine therapy when they received median 48 units of RBC (range: 18-164). Eight patients already had complications (liver cirrhosis: 2, cardiomyopathy: 2, diabetes mellitus: 4) at the time of starting deferoxamine. Eleven patients received treatment for underlying disease on a curative intent (allogeneic stem cell transplantation, 7; autologous transplantation, 1; combination chemotherapy, 3), but only three of them responded. Four patients died of underlying diseases and three patients died of complications associated with treatment for underlying disease. Two patients died of cardiomyopathy. Median overall survival after the diagnosis of underlying disease was 150 months. Iron overload is a common complication of adult chronic refractory anemia. The risk of developing serious complications increased with the increase of RBC transfusions. Patients who developed cardiomyopathy had a worse prognosis especially and therapy should be started earlier to prevent associated complications.

1059
FERRETN LEVELS, NON-COMPLIANCE AND ADVERSE EVENTS IN RELATION TO INFUSED IRON CHELATION THERAPY IN AN INTERNATIONAL COHORT OF PATIENTS FROM ACTUAL PRACTICE
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Background. Deferoxamine (DFO) is an iron chelation therapy (ICT) administered to patients undergoing chronic blood transfusions. Although efficacious, it is burdensome to patients because of the necessity of almost daily infusions lasting 8 to 10 hours and the occurrence of treatment related adverse events. Non-adherence to ICT, however, results in iron overload which, if not removed, results in serious clinical and economic outcomes such as myocardial, endocrine and hepatic dysfunction. Aims. To document ferritin levels, non-compliance and prevalence of adverse events in a cohort of patients undergoing infused ICT. Methods. A retrospective, semi-prospective study of the economic and quality of life burden of infused ICT in the usual care setting was undertaken. Compliance and adverse events were obtained from patient interviews. Serum ferritin level data and adverse events experienced by these same patients during their initial and most recent year of ICT therapy were collected from the patients' medical charts. Results. 78 patients (44% male; mean age: 28.9 ± 14.6 years) with thalassemia (n=51), sickle cell disease (n=23), and myelodysplastic syndrome (n=4) were recruited from 8 different sites within the US (4 sites) and the UK (4 sites). Sixty per cent of patients reported non-compliance to ICT over the previous year. Of these, 43% could be considered at risk for iron overload complications because they reported missing doses more than 2 out of 5 doses. Over the previous 30 days, 64% of patients suffered at least one adverse event; those most commonly reported were site soreness (86%), site irritation (74%), ringing in the ears (22%) and abdominal pain (20%). Of the 56 (75%) patients who had missed at least one dose during the past 4 weeks, 23% did so because of adverse events to ICT. During the initial year of ICT, the adverse events documented in the charts of 8 patients were injection site soreness/rash (30%), allergic reaction to medication (13%), breathing problems (13%) and nausea (13%) while in the most recent year of ICT, the adverse events were commonly reported by 15 patients were site soreness/rash (31%), tinnitus (13%) and joint pain (13%). Serum ferritin level test results obtained from charts indicate that, in general, average blood iron levels are somewhat high and increase slightly over time despite ICT. In some patient categories, this increase is more pronounced. For the initial year of ICT, the mean serum ferritin level was 2,618±1,577 ng/mL (US:2,519±1,382, UK:3,013±1,370) and 2,766±2,272 ng/mL (US:2,741±2,532, UK:2,813±1,740) for the most recent year. For patients for whom data of the most recent year and the first year of ICT were available, the mean serum ferritin level increased by 394.1± 2,633 ng/mL (US:2,741±2,532, UK:2,813± 1,740) for the most recent year. For patients for whom data of the most recent year and the first year of ICT were available, the mean serum ferritin level increased by 394.1±2,633 ng/mL (US:2,741±2,532, UK:2,813±1,740) for the most recent year. For patients for whom data of the most recent year and the first year of ICT were available, the mean serum ferritin level increased by 394.1±2,633 ng/mL (US:2,741±2,532, UK:2,813±1,740) for the most recent year. For patients for whom data of the most recent year and the first year of ICT were available, the mean serum ferritin level increased by 394.1±2,633 ng/mL (US:2,741±2,532, UK:2,813±1,740) for the most recent year. For patients for whom data of the most recent year and the first year of ICT were available, the mean serum ferritin level increased by 394.1±2,633 ng/mL (US:2,741±2,532, UK:2,813±1,740) for the most recent year. For patients for whom data of the most recent year and the first year of ICT were available, the mean serum ferritin level increased by 394.1±2,633 ng/mL (US:2,741±2,532, UK:2,813±1,740) for the most recent year.
**1060**

**RESPONSE RATE IN IDIOPATHIC THROMBOCYTOPENIC PURPURA: A SINGLE CENTER EXPERIENCE**

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**Backgrounds.** Idiopathic thrombocytopenic purpura (ITP), also referred as immune or autoimmune thrombocytopenic purpura, is an acquired disease characterized by low platelet count, normal bone marrow, usually with an increased number of megakaryocytes and the absence of any other disease. The majority of patients respond, on short term, at an initial corticosteroid therapy, but this approach does not influence the natural evolution of the disease on long term, as only about a third of the patients remain in sustained remission at the cessation of treatment. Aims. The study analysed retrospectively the therapeutic response in patients with ITP followed in our institution between 1996 and 2005. **Method.** A precise number of test was done to all thrombocytopenic patients. After the differential diagnosis a positive ITP diagnosis was established in 43 patients, 34 women and 9 men. They were treated commonly with various doses of corticosteroids. Other administered treatments included intravenous immunoglobulin (IVIg), splenectomy, vinca alkaloids, platelet concentrate and rituximab. We consider a sustained response a platelet count above 50,000/µL or above 30,000/µL without hemorrhages or only with minor purpura. A complete response was considered a platelet count above 150,000/µL after the discontinuation of therapy. **Results.** The mean age of the patients at diagnosis was 35,68±16,44 years, (range, 9 and 74). The mean time from diagnosis was 26,69 months (0-288), with 19 new cases. The mean platelet count before treatment was 22,54±22,856/µL, with limits between 1,000 and 117,000. The initial treatment consisted of oral prednisone or methylprednisolone, 1-2 mg/kg/day. High-dose therapy consisted in 40mg dexamethasone by intravenous infusion (iv) or 250-100mg methylprednisolone iv, both per day, for 4 consecutive days. Splenectomy was consider after 3 to 6 months in patients resistant to corticosteroids or earlier at patient demand. The response to corticosteroids, appreciated by the raise of platelets count, in the 40 patients treated this way, was obtained in 25 cases (62,5%), was partial in 5 cases and in 10 patients there were no response. The complete response to corticosteroids was sustained only in 6 cases (15%). In other 11 cases the response was sustained but the platelet count was well below 150,000 (response rate 39,53%). In 10 cases splenectomy was done and 4 cases attained remission, in 4 cases the dose of prednisone needed decreased and in 2 cases no effect was observed. There was only one death (15 years after diagnosis) in our study (2,52%) and no severe infection in patients with splenectomy. **Conclusions.** The diagnosis of ITP covers a large spectrum of patients, with low mortality, but with important morbidity and treatment difficulties. Response to corticosteroids was as predicted. Splenectomy was curative in only 40% of patients previously resistant to corticosteroids. A complete response to corticosteroids was observed repeatedly in our single fatal case.

**1061**

**TREATMENT OF THROMBOTIC THROMBOCYTOPENIC PURPURA: A SINGLE CENTER EXPERIENCE**

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**Background.** TTP is a severe syndrome, often with a subtle beginning, characterized by several clinical manifestations as thrombocytopenia, microangiopathic hemolytic anemia, fleeting neurological signs, renal failure and fever. These clinical manifestations are due to the formation of thrombi rich in platelets in the microcircule with consequent tissue ischemia. Pregnancy, infections (E. Coli and Entherohaemorragic 0157) and neoplasia may represent triggering factors. In some cases an important pathogenic role is represented by a constitutional or acquired (autoimmune) deficiency of a metalloproteinase, ADAMTS 13. The inadequacy of ADAMTS 13 (A Desintegrin And Metalloprotease with ThromboSpondin type I domain) family. This metalloproteinase deficiency affects the procoagulant degradation of VWF (Von Willebrand Factor) multimers induc-
child did not finish the treatment due to allergic reaction. Two other children did not respond. Three patients with AIA has been in CR for 1 to 2.4 years and ongoing. One patient (21 years-old) had CR for only one month. The patient with Evans syndrome has been in CR for 14 months and ongoing. Therapy was well tolerated, except for an allergic reaction in two patients, and no infectious complications occurred. Steroids were withdrawn in patients in CR. The CD20+ B cell count decreased in most patients to less than 1% after Rituximab. Patients with refractory chronic immune cytopenias respond well to Rituximab, even after splenectomy. Rituximab may be considered before splenectomy in patients with high risk of complication, and it allows to withdraw steroids when the patient enter in CR.

1063 QUANTIFICATION OF SEMINAL THROMBOMODULIN
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Backgrounds. Semen forms a gel-like-coagulum immediately after ejaculation, embracing the sperm. Subsequently semen liquefies spontaneously. The presence of fibrin degradation products, prothrombin fragments, and other active components of the plasma clotting system in seminal plasma have previously been reported. Aim: To investigate the presence of thrombomodulin in human semen. Materials and Methods. Using an ImubindTM Thrombomodulin ELISA assay - seminal thrombomodulin antigen levels were measured in 37 semen specimens obtained from sub-fertile, normally fertile, fertile sperm donors and vasectomized subjects. Results. Thrombomodulin was quantifiable in human semen. The vasectomy group showed the lowest value. Slightly higher levels were seen for the normal, fertile sperm donors and the pooled normal semen parameters stratification group (derived from the World Health Organization [WHO] fertility criteria) compared to the infertile subjects. However, there were no significant differences between these groups when tested against each other. Seminal thrombomodulin levels showed negative association with total sperm concentration (density), sperm counts per mL, days of abstinence, liquefaction time and semen volume. Conclusion. Our results establish the presence of thrombomodulin in human semen. Thus, provide further evidence for some involvement of the conventional haemostatic system in the coagulation and liquefaction properties of human semen.

1064 CLONAL PATTERNS OF HEMATOPOIETIC STEM CELLS IN PERIPHERAL BLOOD OF PERSONS ACCIDENTALLY EXPOSED TO HIGH DOSES OF RADIATION
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Background. The polyclonality of hematopoiesis was revealed using individually marked hematopoietic stem cells (HSC) in mice, dogs and primates. The insertion site analysis of human hematopoietic cells engrafted in immune-deficient mice identified several individual clones that contributed to hematopoiesis. However, the relevance of these xenograft models to natural human hematopoiesis in vivo remains unclear. HSC of persons exposed to high doses of radiation bear stable chromosome aberrations during whole life. Aim: The dynamics of HSC functioning in vivo is poorly understood because of the difficulty in clonally tracking individual stem cells. The goal of this study was to investigate clonal contribution to hematopoiesis in humans. Methods. Stable chromosome aberrations in individual HSC were used to evaluate the fate of distinct human hematopoietic clones bearing unique chromosome markers in peripheral blood cells in persons after high-dose irradiation and consecutive hematological recovery. Clonal chromosome aberrations were evaluated in individual colonies in semisolid media developed from peripheral blood cells and in PHA-stimulated lymphocytes. Five healthy donors and 7 persons after in vivo exposure to 1,9-5,4 Gy from 2 to 48 years before investigation were studied. There were no clonal chromosome aberrations in bone marrow cells, peripheral blood lymphocytes and monocytes of donors derived from healthy donors. Results. Clonal chromosome analysis of individual colonies was performed in 5 cases of irradiated persons. Frequency of colonies with unique clonal markers varies from 0 to 100% depending on exposure doses. In patients 1 and 2 (exposure dose - 3,8 and 5,4 Gy) colonies with different clonal markers were found, the same unique clonal chromosome markers were sometimes revealed in 2-3 colonies. During 2003-2005 years patient 2 was analyzed three times repeatedly and no colonies with the same aberration were found. Patient 3 (exposure dose - 3,6 Gy, 20 years ago) was analyzed only once and all available colonies showed bearing the same marker looking as del(12)(q21) or t(c;12)(translocation t12;q21) could not be excluded. In the other two patients (exposure dose - 1 and 2,3 Gy) colonies with clonal chromosome markers were not found. In peripheral blood lymphocytes of 4 patients stable chromosome aberrations were found in 22-55% of cells depending on irradiation dose. In two of them 9-15% of aberrations were clonal. From 4 to 8 clones were found in each case, some of the clones were large and represented 2-5% of evaluated cells. The indication of stable chromosome aberrations in T-lymphocytes can be explained by both a direct radiation effect on long-living lymphocytes and indirect cell defects originating from HSC. Summary/Conclusion. Our preliminary results suggest that hematopoiesis in humans is polyclonal. The size of the clones, their longevity and kinetics will be the subject of further investigation.

1065 EXCELLENT STEM CELL MOBILIZATION USING ESCALATED BEACOPP IN HIGH-RISK PATIENTS WITH HODGKIN’S DISEASE
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Introduction: After intensive treatment regimes have been established, the survival rate for patients with advanced Hodgkin’s disease is approximately 91% after five years and 15% of the patients have a relapse or have primary progressive disease (2%) within the first five years. For patients with relapse after conventional chemotherapy + radiotherapy, however, there is a real chance of achieving remission again. Since it is often difficult to harvest autologous stem cells following an intensive pre-treatment, our center embarks on the strategy to harvest autologous blood stem cells in high-risk patients, defined according to the risk stratification of the German Hodgkin’s Study Group, already as part of the initial polychemotherapy. Results. Between 9/2003 and 2/2006, we analyzed the results of the stem cell harvest of 14 consecutive patients with Hodgkin’s disease who were mobilized with the escalated BEACOPP regimen. There were 9 female and 5 male patients. Escalated BEACOPP was the primary therapy in twelve patients and a relapse was treated in two patients; the previous treatment was 4 or 6 cycles of the ABVD regime + involved field radiation. The twelve patients who did not receive previous treatment were classified as having an initial Ann Arbor stage IIA/2, IIB/5, IIIA/3 and IVB/2 and most of them had a large mediastinal bulk as an additional risk factor. The two patients who did receive a previous treatment were classified as having an initial Ann Arbor stage IIA or IIB, without an additional risk factor. The stem cells were collected in 1 patient from cycle 2, in 9 patients from cycle 3 and in 4 patients from cycle 4 of the escalated BEACOPP regimen. A total of 13 patients received a standard dose of filgrastim, 5 µg/kg body weight s.c., from day 8 up to the apheresis and 1 patient received pegfilgrastim 6mg s.c. All aphereses were performed using an Amicus cell separator™ (Baxter, MNC set, closed two-arm). 9 patients required only 1 apheresis and the remaining 5 patients required 2 aphereses. An apheresis result sufficient for a possible reinfusion could be achieved in all patients (4.26 - 14.4 x 10^6 CD34+ cells/kg/body weight, mean: 7.7). Summary. According to our experience, escalated BEACOPP regimen is very suitable for the harvest of stem cells in high-risk patients with Hodgkin’s disease even though the stem cells have acquired sufficient quantities to also be collected from pretreated patients. The stem cell mobilization can be integrated into the escalated BEACOPP regimen safely and without a delay in treatment and thus creates, already at an early stage, the precondition for a high-dose therapy, which might be required in high-risk patients.

1066 MARROW CELLS CULTURED IN MSC MEDIUM EXPAND TO CD37, CD99 AND CD105 CELLS OF FIBROBLAST-LIKE MORPHOLOGY
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Backgrounds. Recent literature data suggest that in the marrow reside progenitors with a potential to regenerate not only hematopoietic sys-
HUMAN BONE MARROW ADIPOCYTES AND HEMATOPOIESIS: FROM UNILOCULAR FAT CELLS TO FIBROBLAST-LIKE FAT CELLS AND THEIR RELATION WITH CD34+ PROGENITORS DIFFERENTIATION IN THE ABSENCE OF EXOGENOUS CYTOKINES

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Backgrounds. The bone marrow microenvironment plays a critical role in regulating the growth and differentiation of hematopoietic cells. Both growth factors and cytokines, as well as direct cell-cell contacts, participate in these processes. Fat cells are heterogeneously present in the bone marrow and replace hematopoietic cells in bone marrow failure disorders. Thus, it is usually admitted that they play a passive role in hematopoiesis. Aims. In this work we have tested the hypothesis that adipocytes could play an active role in hematopoiesis.

Methods. Adipocytes isolation, cell culture, RT-PCR, optic cytology, electronic microscopy, immunophenotypic analysis (FACS, confocal) and ELISA. Results. cocultures of FLFC and CD34+ positive cells, induce CD34+ differentiation essentially into macrophages (Mo) and dendritic cells (DC). Likewise, FLFC and CD34+ cells in co-culture can produce SCF, M-CSF and GM-CSF. In contrast, granulopoiesis was poorly represented and erythropoiesis was totally inhibited even in the presence of high dose of Erythropoietin (2U/mL). FLFC establish cell-cell contacts with Mo and DC, but this contact is however not critical since DC and Mo can be obtained in a transwell coculture. In contrast, in transwell experiments erythropoiesis and granulopoiesis were restored. Summary/Conclusion: Our data suggest that adipocytes have an active role in hematopoiesis. They may induce CD34+ cells differentiation towards Mo and DC, and inhibit through cell-cell contact, erythropoiesis and granulopoiesis. Our data may provide a new role for adipocytes in vivo, and may actively participate at the pathophysiology of bone marrow failure disorders. It remains to determine by which mechanisms FLFC operate in this process, to define new therapeutic strategies able to target FLFC.
Sensitization of Glioblastoma Cells for Death Receptor- or Anticancer Drug-Induced Apoptosis by PI3K Inhibition

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Activation of the PI3K/Akt/mTOR pathway has recently been reported to correlate with increasing tumour grade, decreased apoptosis and adverse clinical outcome in human malignant glioma in vivo. However, the therapeutic potential of targeting the PI3K/Akt/mTOR cascade by kinase inhibitors for apoptosis sensitization of malignant glioma has not yet been investigated in detail. Here, we report that inhibition of PI3K by LY294002 significantly sensitized glioblastoma cells for death-inducing ligands (TRAIL, agonistic anti-CD95 antibodies) as well as for different anticancer drugs (Doxorubicin, Taxol, Vincristin). In contrast to PI3K inhibition, blockade of mTOR by RAD001 (everolimus) or of MEK by UO126 did not significantly alter the sensitivity of glioblastoma cells for TRAIL- or Doxorubicin-induced apoptosis. Analysis of apoptosis pathways revealed that inhibition of PI3K resulted in downregulation of anti-apoptotic proteins such as FLIPs, XIAP, cIAP2 and survivin and cooperated with TRAIL or Doxorubicin to trigger loss of mitochondrial membrane potential, release of cytochrome c from mitochondria and full activation of the caspase cascade. Inhibition of caspases by the broad range caspase inhibitor ZVAD.fmk completely abolished apoptosis in response to combined treatment with LY294002 and TRAIL or Doxorubicin, indicating that apoptosis occurred in a caspase-dependent manner. By demonstrating that inhibition of PI3K significantly enhanced both death receptor- and anticancer drug-induced apoptosis in glioblastoma cells, our findings have important implications for the development of novel treatment strategies in glioma therapy. Thus, PI3K inhibitors represent a promising approach to enhance the antitumor activity of TRAIL or chemotherapy in glioblastoma.

HBV-Var-XPRbase: A Comprehensive Online Repository of Experimental Protocols to Screen for Human Globin Gene Sequence Variations

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Backgrounds. Hemoglobinopathies, resulting from mutations in the α- or β-like globin gene clusters, are the most common inherited disorders in humans, with approximately 7% of the world population being carriers of a globin gene mutation. Single nucleotide substitutions can lead to amino acid replacements that cause hemolytic anemias, such as sickle cell disease, or hemoglobinopathies that are unstable or have altered oxygen affinity. Molecular defects in either regulatory or coding regions of the human α-, β-, or δ-globin genes can minimally or drastically reduce their expression, leading to α-, β- or δ-thalassemia, respectively. Other sequence changes have little or no effect on hemoglobin function, but are useful polymorphisms for genetic studies. A plethora of mutation detection methods are currently available for human globin gene mutation analysis. Recently, Kollaris et al. (2009), established the database HbVar (http://globin.cse.psu.edu, Hudson et al., 2002), has been developed by a multi-center academic effort, in order to provide globin research community with (i) up-to-date and high quality information on the genomic sequence changes leading to hemoglobin variants and hemoglobinopathies, (ii) globin gene mutation frequencies in various populations (Patrinos et al., 2004) and (iii) the option to combine information on hemoglobin variants and thalassemia mutations with a wide spectrum of genomic data. Aims. The construction of HbVar-XPRbase (http://www.goldenhelix.org/xprbase), an electronic database aiming at collecting to a single website all the experimental protocols available for mutation screening in the human globin genes. Database contents: HbVar-XPRbase is a curated database, which includes a concise listing of the available globin gene mutation screening strategies. HbVar-XPRbase is a flat-file database and operates under PHP. The available experimental protocols to screen for the different human globin gene mutations and/or polymorphisms have been extracted from the published literature, re-constructed to be more concise and comprehensive and made available for the users to query upon. Information in this database is stored in such way that the user can formulate different queries, for example for the available DGGE protocols for mutation screening in all globin genes, or for the mutation detection technologies only for the δ-globin gene. In addition, links to specific globin gene mutations, stored in HbVar, redirect the user to the mutation in question. In addition to these, all the users that have identified one or more of these mutations have been identified. Emphasis has been given so that the protocols stored within HbVar-XPRbase describe a general mutation detection strategy, spanning across the entire genomic region of a particular globin gene. Conclusions. HbVar-XPRbase provides a comprehensive collection of human globin gene mutation screening protocols, allowing the researchers and diagnostic laboratories to easily choose from a single website, those protocols which suit better to their needs.

Alterations of Gene Expression in PTH (1-34) Treated Long-Term Bone Marrow Cultures from Patients with Aplastic Anemia

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Background. Pathophysiology of aplastic anemia (AA) is not well understood. An impairment of regulation of the hematopoietic potential could be caused by abnormal interaction between AA hematopoietic stem cells and their microenvironment. It is possible to establish stromal layer from AA patients in vitro, but it fails to support healthy hematopoietic stem cells, suggesting a defect in the marrow microenvironment of the patients. Our group has established stromal cell cultures from one of the hematopoietic stem cell niche participants. Administration of PTH leads to increased generation of osteoblasts as well as enhanced osteoblast function. An increase in the number of stem cells was observed in animals after PTH injection, and survival after bone marrow transplantation was markedly improved. PTH treatment of long-term bone marrow culture improved adhesion of stem cells to stromal layers and maintenance of hematopoietic precursor cells. Aims. The aim of this study was to find out if PTH treatment could cause any alterations in expression of some genes in adherent cell layers (ACL) of cultures from AA patients. Methods. Long-term bone marrow cultures were established from 19 patients with aplastic anemia and 24 donors as a control group. PTH was added for 3 and 6 weeks of cultivation in concentration 10-8, 5x10-7 and 10-6 M once a week while changing half of the media. To characterize alterations in the expression of several genes in ACLs after PTH treatment semi-quantitative analysis of RT-PCR products was performed using Phospholmager Cyclone, Packard Bell (USA) after Southern blot hybridization with appropriate sequences. The expression level of β-actin was used as a normalization factor. Results. Osteoblastic cells activated by PTH produced high levels of the Notch ligand Jagged 1. Expression level of Jagged 1 increased insignificantly in donors’ ACLs after PTH treatment and did not change in AA patients’ ACLs. In ACLs of both groups, the expression level of Notch was stable and independent of PTH treatment or duration of cultivation. Expression level of Bmi 1 and Ang 1 genes taking part in regulation of HSC proliferation did not change in PTH-treated cultures from AA patients. Moreover, after 3 weeks in culture expression of Ang 1 was 3-fold lower in these cultures compared with donor ones. In donor ACLs PTH administration caused 3-fold increasing of Bmi 1 expression. Expression levels of cell adhesion molecules VCAM 1 and ICAM 1 in ACLs of both donors and AA patients were not sensitive to PTH treatment while in donor ACLs expression of ICAM increased significantly during cultivation. VEGF expression remained constant in donor ACLs after both PTH treatment and cultivation, whereas no changes in its expression were observed in AA patients ACLs. Summary/Conclusion. Stromal cells from patients with AA are not sensitive to PTH treatment. It may happen due to absence of activation of osteoblastic cells in their microenvironment after PTH administration and may point to pathology of these cells.

Acute Myeloid Leukemia: Outcome of Relapse Following Allogeneic Stem Cell Transplantation Comments on 46 Cases

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11th Congress of the European Hematology Association
Acute myeloid leukemia (AML) patients relapsing after allogeneic stem cell transplantation (allo-Tx) have a very poor prognosis. Discon- tinuation of immunosuppression and donor lymphocyte infusion exhibit- it activity against leukemic cells. However, long term outcome is disappointing. Patients’ selection for salvage treatment is very important. Forty-six consecutive AML patients relapsed after allo-Tx between 9/1992 and 7/2005. Age at transplantation ranged between 15 and 60 years (median, 36). Twenty-six patients were in early, 14 in intermediate, and 19 in advanced stage at the time of transplantation. Donors were HLA identical siblings (n=34), mismatched related (n=6) and matched unrelated (n=6). In all but one cases ablative conditioning was used. In all but one (haploidentical sibling with 5 HLA Ag m/m), cases T-cell replent- ed grafts were given. Relapse occurred 1 to 90 months after transplanta- tion (median, 5). Eighty patients had an early relapse (<30% blasts in BM), and two patients had only extramedullary relapse. Salvage therapy was introduced in 30 of the 46 patients and included: Combined chemotherapy (n=5), donor lymphocyte infusion (n=5), combined chemotherapy or serotherapy plus donor lymphocytes or haemopoietic cells (n=11), high dose cytarabine plus haemopoietic cells (n=9). One patient suffering from acute promyelocytic leukemia was treated with As2O3 among other modalities. Eleven patients died due to ther- apy related toxicity (37%). Complete remission was achieved in 15 patients (50% of the patients receiving salvage therapy and 58% of the total relapsed patients). Four patients are alive and in CR for 3, 22, 40, and 53 months after relapse. In two out of these four patients acute and subsequently progressive chronic graft versus host disease developed for the first time after relapse. Overall survival for all patients is 12.7% at 4 years. Among eight patients with early relapse, 5 are alive and in CR for 3, 22, and 53 months. Both patients with extramedullary relapse are alive but still with relapsing extramedullary disease for 14 and 99 months. In conclusion, the very poor prognosis of relapse after allo-Tx in AML is confirmed. Even in selected patients salvage therapy is accom- panied with high mortality rate. Nevertheless, it must be emphasized that in a small proportion of patients long and disease-free survival is achieved. A possible survival advantage of patients with early relapse (i.e. ≥30% blasts in BM) points out to the importance of close follow- up after Tx. Long survival (even not disease-free) of patients with extramedullary relapse is remarkable and the involved mechanisms deserve further studies.

1075 USE OF FROZEN EMBRYOS FOLLOWING STEM CELL TRANSPLANTATION FOR LEUKEMIA: A SINGLE CENTRE EXPERIENCE.

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Infertility is common following stem cell transplantation (SCT) for leukemia. Options for parenthood need to be considered before high dose chemotherapy and total body irradiation are given and include cryo- preservation of fertilised embryos, oocytes (unfertilised mature eggs) or ovarian tissue. The pregnancy extraembryonic transfer in otherwise healthy women is approximately 25% with a take home baby rate of approximately 16-18%. The success rate using frozen embryos in women who have received chemoradiotherapy as treatment for cancer is not known. There are theoretical concerns that success will be lower in these patients compared to the normal population because of the effects of chemoradiotherapy on the uterus. Pre-transplant chemother- apy or disease may also have deleterious effects on the female reproductive system. At the IVF Unit, Hammersmith Hospital, 6 women with an underlying diagnosis of CML have attempted pregnancy using embryos cryopreserved prior to SCT.

Table 1. Outcome of frozen embryo transfer.

<table>
<thead>
<tr>
<th>Treatment cycles</th>
<th>Pregnant/ not pregnant (NP)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1 n=3</td>
<td>P</td>
<td>miscarriage</td>
</tr>
<tr>
<td>Patient 2 n=1</td>
<td>P</td>
<td>Live birth</td>
</tr>
<tr>
<td>Patient 3 n=1</td>
<td>NP</td>
<td>–</td>
</tr>
<tr>
<td>Patient 4 n=2</td>
<td>NP</td>
<td>–</td>
</tr>
<tr>
<td>Patient 5 n=2</td>
<td>P</td>
<td>miscarriage</td>
</tr>
<tr>
<td>Patient 6 n=2</td>
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<td>–</td>
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</tbody>
</table>

Of these 6 women, 5 had allogeneic SCT with conditioning which included total body irradiation and one had an autologous SCT condi- tioned with high dose busulphan only (patient 4). The results are tabu- lated below. Eleven treatment cycles using cryopreserved embryos in 6 women led to 4 pregnancies in 3 women. Of these, two pregnancies have been successful. There have been two miscarriages, one (patient 1) at 12-13 weeks gestation and the second (patient 5) at 7 weeks gestation. The success rate using frozen embryos is approximately 16-18%. The success rate using frozen embryos in 6 women led to 4 pregnancies in 3 women. Of these, two pregnancies have been successful. There have been two miscarriages, one (patient 1) at 12-13 weeks gestation and the second (patient 5) at 7 weeks gestation. The success rate using frozen embryos is approximately 16-18%. The success rate using frozen embryos in 6 women led to 4 pregnancies in 3 women. Of these, two pregnancies have been successful. There have been two miscarriages, one (patient 1) at 12-13 weeks gestation and the second (patient 5) at 7 weeks gestation. The success rate using frozen embryos is approximately 16-18%. The success rate using frozen embryos in 6 women led to 4 pregnancies in 3 women. Of these, two pregnancies have been successful. There have been two miscarriages, one (patient 1) at 12-13 weeks gestation and the second (patient 5) at 7 weeks gestation. The success rate using frozen embryos is approximately 16-18%. The success rate using frozen embryos in 6 women led to 4 pregnancies in 3 women. Of these, two pregnancies have been successful. There have been two miscarriages, one (patient 1) at 12-13 weeks gestation and the second (patient 5) at 7 weeks gestation. The success rate using frozen embryos is approximately 16-18%.
ANGIOGENIN LEVELS IN PATIENTS WITH POLYCYTHEMIA VERA

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Angiogenin is a protein with a potent function in angiogenesis, which circulates in human serum and is secreted by hematopoietic cells, endothelial cells, vascular smooth cells and fibroblasts. Its serum levels are increased in patients with solid tumors, acute myeloid leukemia, myelodysplastic syndromes, chronic myeloid leukemia and essential thrombocythemia. No data are available about angiogenin levels in patients with polycythemia vera. In this study we aimed to evaluate the levels of angiogenin in serum of patients suffering from polycythemia vera and examine any possible correlation with bone marrow microvascular density (MVD) detected by CD34 count on bone marrow trephines. A total of 29 patients with PV (14 males and 15 females) with a mean age of 57 ± 15,4 (m ± SD) years (range 24-81) were included. The control group consisted of 16 healthy subjects (8 males and 8 females) with a mean age of 55.9 ± 6.7 years (range 46-71). Serum levels of angiogenin were measured by a commercial quantitative sandwich enzyme immunoassay. In twenty four of them we estimated the MVD in bone marrow samples immunostained with anti-CD34 monoclonal antibody by counting the number of vessels per 400x high power field (HPF) using light microscopy. Serum angiogenin concentrations were found to be significantly higher in polycythemic patients than in the control group (48±145 pg/mL and 540±94 pg/mL, respectively, p=0.037). In the patient and in the control group we found no statistically significant correlation between serum angiogenin levels and platelet counts, haemoglobin, WBC counts and age. No difference was found between patients angiogenin levels on different therapeutic regimens. The microvessel density of the bone marrow of the 24 polycythemic patients was found to be 7,1±4,1 vessels per HPF and significantly elevated in comparison to normal bone marrow specimens (n=10, MVD: 2,0±0,6, p=0,01). Interestingly a negative correlation was found between serum angiogenin levels and the microvessel density of the bone marrow (r= -0,49, p=0,035). In the present study, serum angiogenin levels were found to be significantly increased in patients with PV in comparison to the control group. To our knowledge there is evidence of pronounced angiogenin in PV by few reports on other angiogenic factors as vascular endothelial growth factor and basic fibroblast growth factor. In addition the current study demonstrated increased MVD in comparison to healthy control group indicating augmented angiogenenic procedure. The observation that MVD is negatively correlated with angiogenin serum levels, although at first sight unexpected, could be explained by the hypothesis that in patients group the less bone marrow vascularity acts modulating the increase of angiogenin production which in its turn enhances the bone marrow vascular expansion. It is emphasized that this observation should be confirmed by other studies as well. The exact contribution of angiogenin in the pathophysiology of PV and its prognostic significance as disease activity marker deserves further research.

HYPERHOMOCYSTEINAEMIA IS ONE OF THE FACTORS OF THROMBOTIC COMPLICATIONS DEVELOPMENT FOR PATIENTS WITH MYELOPROLIFERATIVE DISORDERS


Myeloproliferative disorders (MPD), such as essential thrombocytopenia (ET), polycythemia vera (PV) and idiopathic myelofibrosis (IMF), characterised by clonal proliferation of haematopoietic stem cells, have an elevated risk of arterial and venous thromboembolic complications. Since hyperhomocysteinemia (HHC) is a risk factor of vascular complications, we had investigated the frequency of HHC in these diseases. We had analysed the possible relationship between the elevated level of homocysteine (HC) in blood serum and vascular complications, and the prevalence of the methylenetetrahydrofolate reductase (MTHFR) enzyme mutation and the effect of this enzyme on HC level in blood.

Materials and results. We analysed 61 patients: 39 patients with MPD with thrombotic episodes in medical history and without them, and 22 non-hematological patients with thrombotic episodes in medical history. Among 39 patients (22 females and 17 males, mean age 41 years, range17-63 years) with ET (n=17), PV (n=8), IMF (n=14) the mean level of HC in blood serum were significantly higher (19±1.7 µmol/L) in comparison with 40 donors in control group (12±1.3 µmol/L, p<0.00002). In the group with thrombotic episodes the mean level in the patients with IMF was much higher (26±4.7 µmol/L), than for patients with ET (20±3.0 µmol/L) and PV (23±8.9 µmol/L), p<0.002. Despite of different frequency of alleles 677 of MTHFR gene in patients with MPD, HHC was observed with the same frequency in all groups, which was different from the situation in healthy people. We did not find out significant differences in the prevalence of genotypes (heterozygous, homozygous) in MTHFR gene in patients with MPD and donors: 72% (8/11) with thrombosis and 42% (8/19) without thrombosis, and accordingly 60% (24/40) in donors population. We did not find out a significant relationship between MTHFR genotype and the rate of thrombotic complications. For MPD patients with normal and elevated HC concentration in blood serum it was shown, that factor VIII level was higher in HHC, than in patients with normal HC level (222±26,5% and 116±20%, p=0,002). The same was found for von Willebrand factor (262±15,6, 120±14,6%, respectively, p=0,008). Comparing the data on ADP induced platelet aggregation in patients with thromboses, it was shown that the activation of the later was higher in HHC against normal data of blood HC (77±15,7% and 47±12,1%, accordingly, p<0,001). Regessenger analyses showed that only HC has a statistically significant influence on thrombotic complications rate for MPD patients (p=0,004). We consider that the lowering of HC plasma levels together with vitamin therapy were the more expressed, the higher was its baseline level. Conclusion. The relationship between HHC levels and thromboses in the patients with MPD was shown. We suppose that different stages of proliferative diseases may influence the HC levels in patients with MPD. Homocysteine is a highly important independent risk factor of thrombotic complications. Therefore it is necessary to discover and treat HHC.

HYPERHOMOCYSTEINAEMIA IS ONE OF THE FACTORS OF THROMBOTIC COMPLICATIONS DEVELOPMENT FOR PATIENTS WITH MYELOPROLIFERATIVE DISORDERS


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Myeloproliferative disorders (MPD), such as essential thrombocytopenia (ET), polycythemia vera (PV) and idiopathic myelofibrosis (IMF), characterised by clonal proliferation of haematopoietic stem cells, have an elevated risk of arterial and venous thromboembolic complications. Since hyperhomocysteinemia (HHC) is a risk factor of vascular complications, we had investigated the frequency of HHC in these diseases. We had analysed the possible relationship between the elevated level of homocysteine (HC) in blood serum and vascular complications, and the prevalence of the methylenetetrahydrofolate reductase (MTHFR) enzyme mutation and the effect of this enzyme on HC level in blood. Materials and results. We analysed 61 patients: 39 patients with MPD with thrombotic episodes in medical history and without them, and 22 non-hematological patients with thrombotic episodes in medical history. Among 39 patients (22 females and 17 males, mean age 41 years,
gene in patients with MPD, our study suggests that the presence of the JAK2 genotype and the rate of thrombotic complications for MPD patients with normal and elevated HC concentration in blood serum was shown. The activation of the later was higher in HC against normal data of blood HC (77 ± 16.7% and 47 ± 21.3%, accordingly, p < 0.01). The same was found for von Willebrand factor (202 ± 15.6, 120 ± 14.6%, p < 0.005). Comparing the data on ADP induced platelet aggregation in patients with thromboses, it was shown that platelet aggregation in patients with thromboses, it was shown that the platelet aggregation in patients with thromboses, it was shown that the activation of the later was higher in HC against normal data of blood HC (77 ± 16.7% and 47 ± 21.3%, accordingly, p < 0.01). The same was found for von Willebrand factor (202 ± 15.6, 120 ± 14.6%, p < 0.005). Comparing the data on ADP induced platelet aggregation in patients with thromboses, it was shown that platelet aggregation in patients with thromboses, it was shown that the platelet aggregation in patients with thromboses, it was shown that the activation of the later was higher in HC against normal data of blood HC (77 ± 16.7% and 47 ± 21.3%, accordingly, p < 0.01). The same was found for von Willebrand factor (202 ± 15.6, 120 ± 14.6%, p < 0.005). Comparing the data on ADP induced platelet aggregation in patients with thromboses, it was shown that platelet aggregation in patients with thromboses, it was shown that the platelet aggregation in patients with thrombo...
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CLINICAL COURSE OF 65 PATIENTS WITH MYELOFIBROSIS WITH MYELOID METAPLASIA: EXPERIENCE OF MONZA CENTER

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**Backgrounds.** Myelofibrosis with myeloid metaplasia (MMM) is a chronic myeloproliferative disorder, characterized by bone marrow reactive fibrosis, extramedullary hemopoesis, progressive anemia and marked splenomegaly. Overall median survival ranges from 3.5 to 5.5 years, according to the presence or absence of adverse prognostic factors, with final evolution toward disease progression (DP) or leukemic transformation (LT). **Aim and Methods.** We analysed 65 MMM patients (pts) referred in our hematology unit from 1999 to 2005, in order to provide information about initial features, treatment, clinical course and survival. **Results.** The median age at diagnosis was 66 years (range 39-79) with 17 pts (26%) aged less than 55 years and a M/F ratio of 44/21. 39 pts (60%) presented Idiopathic MMM, 26 (40%) MMM secondary to Polycythemia Vera (7) or Essential Thrombocythemia (19). At diagnosis, spleen enlargement (median 5 cm, range 1-30) below costal margin was present in 52 pts (80%). The median value of WBC was 11,9×10^9/L, of Hb was 11,6 g/dl, of platelets was 558×10^9/L. 45 pts (74%) had circulating myeloid precursors; 21 (32%) pts had blasts. The median value of LDH was 931 U/L and the median count of CD34+ cell was 59,8×10^9/L (evaluated on 37 pts). According to disease status, 15 pts (23%) received no treatment, 13 (20%) supportive care alone, 9 (14%) androgens or steroids, 23 (35%) anti-platelet drugs and 27 (41,5%) myelosuppressive agents or in combination with the above treatment. Eight pts (12%) underwent splenectomy after a median of 11,5 months from diagnosis. Only 3 pts underwent allogeneic stem cell transplantation. 50 pts (77%) are actually alive after a median follow-up of 28 months (range 3-84). According to Dupriez scoring system, 4 pts (6%) were assigned to high risk (HR), 25 (38,5%) to intermediate risk (IR) and 36 (55,2%) to lower risk (LR) group. The median survival was 14, 24 and 34 months for HR, IR and LR group, respectively. The median survival in pts aged less than 55 years was longer (38 months) with significant difference between LR (59 months), IR (28 months) and HR (15 months) pts. Fifteen pts (23%, 3 pts aged less than 55 years ) died, 6 for LT or DP, 4 for thrombotic or bleeding, 3 for secondary cancer and 2 for heart failure. At time of LT and DP, most pts worsened organomegaly and constitutional symptoms and presented higher LDH levels than at diagnosis. **Conclusions.** Our experience confirms different outcome of MMM pts according to Dupriez score. Younger patients have a longer median survival. DP and LT correlate with increase of spleen volume, onset of constitutional symptoms and higher LDH levels respect to diagnosis.

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THE CD44 MAAB A3D8 INDUCES APOPTOSIS AND G1 CELL CYCLE ARREST IN NEOPLASTIC MAST CELLS IN SYSTEMIC MASTOCYTOSIS

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**Backgrounds.** Recent data suggest that CD44 may serve as a new therapeutic target in AML and possibly also in other myeloid neoplasms. Systemic mastocytosis (SM) is a myeloid neoplasms characterized by abnormal growth and accumulation of mast cells (MC) in one or multiple organs. We have previously shown that normal tissue MC express CD44. **Aims.** In the present study, we asked whether CD44 is expressed on neoplastic human MC and whether CD44 ligation by the monoclonal anti-body (mAb) A3D8 would be associated with inhibition of growth of neoplastic MC. **Methods and Results.** As assessed by flow cytometry, primary neoplastic MC were found to express CD44 in all patients with SM analyzed (n=10). The human mast cell leukemia (MCL) cell line HMC-1 was also found to express CD44. As assessed by ‘H-thymidine incorpora-tion, the CD44 mAb A3D8 decreased the proliferation of HMC-1 cells in a dose-dependent manner (A3D8, 5 µg/mL: 46±26% of control=100%, p<0.05). Similar effects of A3D8 were observed with primary neoplastic MC obtained from a patient with MCL (A3D8, 5 µg/mL: 68±20% and one with smouldering SM (A3D8, 2.5 µg/mL: 42±7% of control, p<0.05). To analyze the mechanism of A3D8-induced growth inhibition, cell sur-vival and cell cycle distribution were analyzed. In these experiments, CD44-ligation induced an approximately 3-fold increase in apoptotic HMC-1 cells compared to control. As assessed by flow cytometry, we were also able to demonstrate that A3D8 induces cell cycle arrest in the G1-phase. **Conclusions.** In summary, our results suggest that CD44-ligation is followed by inhibition of growth of neoplastic human MC through induction of apoptosis and G1 cell cycle arrest. Whether targeting of CD44 in neoplastic MC in patients with high grade MC disorders is of clinical significance remains to be determined in forthcoming studies.

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SYSTEMIC MASTOCYTOSIS WITH EOSINOPHILIA (SM-EO): CLINICAL SIGNIFICANCE OF MOLECULAR MARKERS AND ORGANOPATHY

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**Backgrounds.** In a group of patients with systemic mastocytosis (SM), marked and sustained eosinophilia is detectable (SM-eo). Although the molecular defect has been defined for some of these patients, little is known about the impact and clinical correlates of eosinophilia in SM. Methods. In a cohort of 61 patients with SM, we identified 11 with per-manent eosinophilia (>1.5 10^9/L). According to the WHO-classification, 4 had indolent SM (ISM), 1 smouldering SM (SSM), 2 SM with associ-ated chronic eosinophilic leukemia (SM-CEL), and 4 aggressive SM (ASM). Results. In the 2 patients with SM-CEL, the FIP1L1/PDGFRα fusion gene-product was detectable, but no KIT mutation at codon 816 was found, whereas in most other SM-eo patients, KIT D816V, but not FIP1L1/PDGFRα, could be detected. Other molecular defects including BCR/ABL, CBFB/MYH11, JAK2 V617F, or a monoclonal T cell receptor rearrangement were not detected in patients with SM-eo. In the two patients with SM-CEL, fatal organopathy of the heart developed. By contrast, in all other SM-eo patients, organopathy, if recorded, affected the bone marrow, liver, or skeletal system, but did not affect the heart, even if eosinophilia persisted for many years. Conclusions. Our data show that the biochemical basis of eosinophilia in SM is variable and correlates with organopathy. SM-eo thus is a prediagnostic checkpoint but not a final diagnosis. For correct final diagnosis and selection of target-ed drugs, it is important to apply molecular markers including FIP1L1/PDGFRα in SM-eo.

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DIAGNOSTIC AND CLINICAL RELEVANCE OF CHRONIC MYELOPROLIFERATIVE DISORDERS PERIPHERAL CELLS ANTIGENS

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**Backgrounds.** Immunophenotype of peripheral blood cells in patients with chronic myeloproliferative disease (CMD) has not been extensively investigated to recognise association between CMD subtype , phase of disease and peripheral cells antigens. **Aim.** The aim of our study is the identification of same cytofluorimetry parameters useful to follow up patients with CMD. Methods. We analyzed the immunophenotype of 20 consecutive patients observed in our institute, during cytoreductive treatment for CMD. Of 20 patients 9 had essential thrombocythemia (ET), 7 myelofibrosis (MF) and 4 atypical myeloproliferative disorders (AMD). Flow cytometry was performed on peripheral blood sample using double or triple platform assay to identify CD34, CD33, CD61, CD42a positive and CD34/33 coexpression on CMD patients cells. Results. The main hematologic characteristics analyzed in CMD patient about white blood cells and platelet count range were in ET group WBC 6.9-16.1×10^9/L, Plt 487-1976×10^9/L, in AMD group WBC 4.26-9.8×10^9/L, Plt 171-387×10^9/L, in MF group WBC 8.53-26.47×10^9/L. The median of circulating CD34, CD33, CD61, CD42a, expressed in 106 cells were in ET group (20, 117.0, 463, 469 respectively), in AMD group (54, 79, 4, 553, 435 respectively), in MF group (63, 261, 33, 145, 371 respectively). We observed higher expression of CD33 than CD34 in CMD patients studied and a significantly elevated median number of circulating CD33 and CD34 in patients with MF, especially not responsive to cytoreductive treatment and inverse relationship between absolute number of CD33 positive and platelet count. Instead we evaluated a sta-tistical correlation between of CD 61 and 42a positive cells and thymocytes in ET and AMD groups than others CMD patients. Summary/Conclusions. These initial data reflect the increased number of circulating CD33 and CD34 in patients with MF, especially not responsive to treatment. This correlation may show an abnormal function of bone marrow for a hematopoietic differentiation decline. Further studies with a larger number of patients could improve the identification of sur-face antigens to classify better subclass of CMD associated to clinical fea-tures and to predict prognostic evolution of disease.

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To assess the benefit/risk balance of long-term anagrelide therapy in patients with ET. Methods. Retrospective analysis of an open-label, multicenter anagrelide trial. Results. Data from 3660 patients with MPD (2251 with ET) were included in the safety analysis. The maximum duration of follow-up was 11.4 years. Prior myelosuppressive therapy had been administered in 81% of patients (reasons for change to anagrelide, toxicity [33%] and poor platelet control [31%]). Efficacy data were available for 954 patients with ET. A response rate of 78.7% was observed (67.2% complete responses [decrease in platelet count to ≤500x10^9/L] or a decrease of ≥50% from baseline within 4 weeks of the start of anagrelide therapy); 11.5% partial response [decrease of 20% to <50% from the baseline value at least 4 weeks after starting anagrelide]. Response rates for patients who had failed previous therapy and for patients who were intolerant of previous therapy were similar (78.8% and 78.6%, respectively). After the first year, platelet count decreases were well maintained. Results were similar for both sexes, different age groupings, and ethnic origins. At baseline, 163/934 (17.5%) patients reported ET-related symptoms, including GI and other bleedings, arterial or venous thromboses, angina, pulmonary embolism, transient ischaemic attacks, peripheral ischaemia, and paraesthesia. This had reduced to 7.9% (63/796, p=0.001) after 12 weeks and was maintained during follow up (3.2% [15/470] at 1 year and 2.5% [6/239] at 2 years, p<0.001). Adverse events occurred in 40.2% of the patients and were generally mild. Anagrelide was discontinued in 38.6% of patients (adverse events accounting for 29.2% of patients stopping treatment). Transformation to AML/MDS occurred in 47/2251 (2.1%), but only in subjects who had never been exposed to cytoreductive treatment. The observed mortality rate (8.8%) was consistent with that which would be expected in ET patients. The most common reasons for death (≥1%) were CML, reason unknown or unspecified, and sepsis. Summary/Conclusions. Anagrelide effectively reduces platelet counts and thrombohaemorrhagic complications in patients with ET. This is independent of gender, age, ethnic origin, and prior therapy. The drug demonstrates a recognized safety profile. Benefits are maintained during long-term follow-up without an increase in disease transformation.

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TREATMENT WITH HYDROXYUREA AS SINGLE AGENT DOES NOT INCREASE THE RISK OF SECOND MALIGNANCIES IN ESSENTIAL THROMBOCYTHEMIA PATIENTS

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Among chronic myeloproliferative disorders, Essential Thrombocythaemia (ET) has the most favourable prognosis, with thrombotic events and second malignancies representing major causes of death. Several agents have been tested to delay or slow down disease transformation. Among these, hydroxyurea is an effective, low toxicity, low cost and easy-to-take drug, without severe side effects.

1089 THE COMBINATION OF FLUDARABINE, Ara-C, IDARUBICIN AND GEMTUMUZUM-OZOGAMICIN (MY-FLA) IS A SAFE AND EFFECTIVE THERAPY FOR ELDERLY AML PATIENTS.

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Background. Elderly AML patients and patients with AML evolved from MDS or therapy related displayed a very poor prognosis. In the last decade the association of fludarabine, Ara-C and anthracyclines proved to be an effective and well tolerated induction regimen for this group of patients and, more recently, the introduction of gemtuzumab ozogamicin has opened new perspectives in the treatment of AML. Methods. We report here our preliminary experience on 18 elderly AML patients treated as first line therapy with MY-FLAI regimen (Fludarabine 25 mg/m², Ara-C 1 g/m², idarubicin 5 mg/m² all for 3 days, followed by gemtuzumab ozogamicin 3 mg/m² at day 4). Responding patients received the same regimen as consolidation therapy. Patients. The median age of patients was 66 (range 54-76); M:F ratio was 8:10; FAB subtypes were M0 in 1 patient, M1 in 8, M2 in 5, M4 in 2, M5 in 1, M6 in 1. Nine patients had de-novo AML (50%); in nine patients AML was secondary to NHL (3), MDS (4), epithelial neoplasms (2). Hematological parameters before therapy were the following: WBC 6x10⁹/L (range 1.7-110); Hb 9.4 g/dL (7.3-12); Plt 40x10⁹/L (15-190). Cytogenetics analysis showed a poor prognosis alteration in 9 patients (50%, with 7 complexes karyotypes) and an intermediate alteration in the other 9 patients. Results. The neutrophil (PMN > 0.5x10⁹/L) and platelet (>25x10⁹/L) recovery required a median of 18 (range 11-23) and 18 days (range 10-29) from the end of therapy. The median number of days with fever (≥38°C) was 5. Therapeutic failures were observed in 6 patients (40%) due to early death (3 patients), disease progression (1 patient), or severe infectious, hepatic or cardiac complications were recorded. The median hospitalization period was 32 days (range 19-56). Eleven pts (60%) achieved CR, 7 (40%) were refractory. Complete remission and
survival had a median length of 6 months (range 2-19) and 8 months (range 3-20), respectively. Five out of 11 patients have relapsed and 9 have died of disease. Poor prognosis cytogenetics had a negative impact on CR rate (44% compared to 77% in patients with intermediate prognosis karyotype), whereas de-novo and secondary AML had similar CR rates (55% and 66%, respectively). Conclusions. Considering the report of outcome of elderly AML patients treated with conventional chemotherapy and the unfavourable clinical features of this series (high percentage of unfavorable karyotype and secondary AML) our preliminary results show that MY-FLA1 may represent a well tolerated and effective induction and consolidation regimen for elderly non M5 AML patients.

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Efficacy and safety of bortezomib in patients with refractory and relapsed multiple myeloma outside clinical trials: results from the Catalan Myeloma/amyloid study group (GEMMAC) in 120 patients
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Background. Bortezomib (Velcade) has recently been approved for the treatment of refractory and relapsed multiple myeloma (MM). In this setting, a response rate ranging from 35 to 50% has been reported in patients included in prospective clinical trials. However, the data on the efficacy and safety of bortezomib outside the context of clinical trials are limited. Aim. To analyze the efficacy and safety of bortezomib therapy in refractory or relapsed MM patients treated in community practice.

Patients and Methods. Between August 2003 and February 2006, 120 patients (63M/57F, median age: 63 years) with refractory or relapsed MM were treated with bortezomib outside the context of clinical trials in 16 centres in the area of Catalonia. Fifty-five (46%) patients had untreated relapse, 38 (32%) refractory relapse and 27 (23%) primary refractory disease. Twenty-seven of them (22%) had extramedullary plasmacytoma. The median number of previous lines of therapy was 2 (range: 1-6). Forty-five patients (42%) had received high dose therapy followed by stem cell transplantation (HDT/SCT); single autologous (55), double autologous (6), autologous followed by allogeneic with reduced-intensity conditioning (6) and allogeneic (3). Bortezomib was administered intravenously at a dose of 1.3 mg/m² on days 1, 4, 8, and 11 of every 21-day cycle. Six patients who had no response after two cycles of bortezomib alone continued treatment receiving also oral dexamethasone. The median number of cycles administered was 3.5 (range: 1-13). At the time of this analysis, bortezomib therapy was still ongoing in 22 cases, and 12 patients were not yet evaluable for response. Responses were evaluated according to the EBMT criteria. Results. Among the 108 already evaluable patients, the response rate to bortezomib was 52% (57/108), with 7 (6%) complete, 58 (55%) partial and 12 (11%) minimal responses. The remaining 51 patients showed no response: no change (18), progressive disease (20), and early death within the first two months from bortezomib onset (13). The median time to best response was 3.5 months (range: 0.5-11). Grade 3 or 4 adverse events, which occurred in 45% of evaluable patients, included: thrombocytopenia (30%), asthenia (12%), peripheral neuropathy (5%), gastrointestinal symptoms (4%), fever (4%), postural hypotension (2%), rhabdomyolysis (1%) and tumour lysis syndrome (1%). The drug was discontinued because of side effects in 10 patients: peripheral neuropathy (7), asthenia (1), thrombocytopenia (1) and unknown (1). After a median follow-up of 7.4 months (range: 1.6-50.4), 15 of the 57 responding patients had relapsed (26%). Conclusion. In this observational study, the response rate to bortezomib in patients with relapsed and refractory MM treated in community hospitals was comparable to that achieved in the recently reported prospective clinical studies. Toxicity was manageable, but led to bortezomib discontinuation in 10% of the patients. In the present series, the evaluation of time to progression and overall survival requires longer follow-up.

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Inherited coagulation disorders in central part of Iran
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Backgrounds. The incidence of hereditary coagulation disorders may vary according to the country and ethnic origin. Demographic datasets are vital in setting priorities, allocation of resources, measurement of outcomes, and comparison of alternate approaches. Aim: The aim of this study was to document the epidemiological features, disease severity and complications associated with inherited coagulation disorders in central part of Iran. Methods. A comprehensive survey was undertaken in January 2006. Clinical history, Laboratory and treatment data, and long term complications of all cases (553 persons) diagnosed with inherited coagulation disorders, were studied in Hematology-Oncology Department, Isfahan University of Medical Sciences. Results. 465 male and 88 female with median age of 29.4±12.9 were studied. Hemophilia A was found in 341(61.7%), 48 (8.7%) had hemophilia B, 74 (13.4%) had Von Willebrand disease, and 34(6.1%) had platelet dysfunctions. The rare coagulation disorders (n=88) include 30 patients with VWF deficiency, 23 with FVIII, 13 with afibrinogenemia, 10 with FX. Among them 19 (9.8%) had combined FVIII and FX deficiency. 228 (41.2%) patients had severe hemophilia. The most common complications were Epistaxis (n=59), Hemartosis (n=51) and Hemophilic Arthropathy (n=49).None of the patients were human immunodeficiency virus positive but 125 (22.6%) were hepatitis C virus positive and 2 (0.4%) were hepatitis B positive. Replacement therapy primarily relied on Cryoprecipitate and Fresh Frozen Plasma due to inhibitor absorption. Conclusion. Most of the hemophilic patients have the severe type of the disease, this differs from that obtained by other studies elsewhere and it may be due to some degree of under diagnosis of the less severe forms of hemophilia. Implement a program of prophylaxis for hemo philic arthropathy in children with severe hemophilia could be helpful. A more stringent policy for blood product usage, HCV screening and HBV vaccination is needed to abolish these diseases in patients with hemophilia.

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Disseminated intravascular coagulation in an angioimmunoblastic T-cell lymphoma
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Introduction. Disseminated intravascular coagulation (DIC) is a syndrome suggested by clinical signs and laboratory tests. The diagnosis may be based on the new ISTH overt-DIC score or other parameters including soluble fibrin monomer complexes (SFMC), antithrombin and protein C consumption. Angioimmunoblastic T-cell lymphoma (AITL) is an uncommon lymphoma. We report the case of a 71-year-old woman who presented AITL with an inaugural DIC associated with additional adverse prognosis parameters like SFMC, antithrombin III (AT) and protein C levels. Clinical observation. This woman was admitted to the hospital for severe health alteration. Clinical examination showed pallor, diffuse enlarged peripheral lymph nodes, hepatosplenomegaly, purpuric vasculitis and bruising. Laboratory analyses showed (~) nadir: hemoglobin 9→7g/dl (nr: 12-16), platelets 64→99×10⁹/L (nr: 150-400), lactic dehydrogenase (LDH) 1272 U/L (nr: 240-480). Coagulation tests were compatible with DIC (ISTH score 5 up to 8): serum fibrinogen was 1.48–4.55g/L (nr: 2-4.5); APTT time was 35→47, PT was 55→87%, D-dimers were up to 9000 mcg/L (nr: 100-500), AT was 42%, 34% (nr: 80-120), D-dimers were up to 12,95–120 mcg/L (normal <30), STA-latest FM, a new immuno-turbidimetric method of fibrin monomer in vitro as a marker of fibrinogen consumption. DIC was compatible with AITL. The patient had a complete response to a multi-agent chemotherapy including doxorubicin, cyclophosphamide, vincristine, mitoxantrone, and dexamethasone. A more rigorous follow-up and a careful evaluation of time to progression and overall survival requires longer follow-up.

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Infiltrating musculo-cutaneous myxoedema in a myelodysplastic syndrome patient
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Objectives. To report a case of infiltrating musculo-cutaneous myxoedema in a 61-year-old patient with myelodysplastic syndrome (MDS) treated with azathioprine and prednisolone.

Methods. A 61-year-old patient with MDS was treated with azathioprine and prednisolone due to disease progression. Three months later, the patient was admitted to the hospital with a 3-month duration of swelling and pain in the arms and legs (Figure 1). Physical examination showed distal swelling of both arms and legs, with tenderness, non-ulcerated skin, and induration of both arms. Routine laboratory investigations showed normal haematology results, normoalbuminaemia, and normal renal function tests. The patient was surface treated by radiotherapy.

Results. Four months later, the swelling started to resolve. At presentation, the patient was healthy and had no pain, tenderness, or discomfort for 4 months. The patient and the family were satisfied with the results and the treatment.

Conclusion. Infiltrating musculo-cutaneous myxoedema is a rare condition in MDS patients. The role of azathioprine and prednisolone is controversial; however, in this patient, it did not lead to myxoedema. This is the first case of infiltrating musculo-cutaneous myxoedema in MDS patients treated with azathioprine and prednisolone.
ical remission with LDH normalisation. Conclusion. AITL is a rare disease. This is reported as a 3rd case described in the literature with a de novo ETV6-RUNX1. DIC was followed by different criteria with a discordant evolution. Indeed, in this case, evolution of coagulation tests and DIC score was not parallel with an early decrease of STA-Liatest FM. More studies are warranted.

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DERANGEMENT OF HEMOSTATIC PROTEINS IN HCV CIRRHOTIC PATIENTS: RELEVANCE TO HEMORRHAGIC DIATHESIS AND THROMBOTIC EPISODES
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Backgrounds. An altered coagulation profile resulting in decreased natural anticoagulant levels leading to haemostatic activation is described in patients with liver cirrhosis. The protein C system, a major physiologic regulator of haemostatic balance, controls thrombin production and guards against thrombotic episodes. Aims. This study was designed to assess the components of protein C system in HCV cirrhotic patients to determine whether alterations in these haemostatic proteins are related to degree of hepatic dysfunction and/or haemostatic activation and development of hemorrhagic diathesis or thrombotic episodes. Methods. Components of protein C (PC) system were assessed in 44 patients with liver cirrhosis. Blood from 15 patients with acute hepatitis, 14 patients with acute liver hematemesis and 14 patients who had portal vein thrombosis (PVT). According to Child-Pugh criteria, all patients were graded Child C. Neutrophil elastase (NE) release was determined by measuring elastase-α1-proteinase inhibitor (E-α1-PI) complex using an immune activation assay. Levels of tumor necrosis factor-α (TNF-α), PC antigen (PC Ag), total protein S (TPS), free protein S (FPS), soluble thrombomodulin (TM), tissue-plasminogen activator (t-PA), t-PA/PAI-1, plasmin-α2-antiplasmin (PA), thrombin-antithrombin III (TAT) and D-dimer (D-D) complexes were measured in plasma by ELISA. Fibrinogen level, functional activities of PC (PC Ft), plasminogen activator inhibitor-1 (PAI-1) and C4b-binding protein (C4b-BP) concentrations were also assessed. Results. Stimulation of the inflammatory process (increased TNF-α, NE and C4b-BP), endothelial injury (elevated TM and t-PA), reduction in anticoagulant proteins (low PC and PS), hypercoagulation and thrombin generation (elevated TAT and D-D), increased consumption (prolongation of coagulation screening tests, thrombocytopenia, hypofibrinogenemia and decreased PC Ft/PC Ag ratio) and accelerated fibrinolysis (increased PA, free t-PA and t-PA/PAI-1 ratio and decreased PAI-1) were detected in different cirrhotic groups compared to controls (15 healthy subjects). The haemostatic defects correlated with the marked elevation of inflammatory mediators and more pronounced (p<0.05) in patients with PVT. A significant decline (p<0.05) in fibrinogen concentration and PC Ft/PC Ag ratio associated with a significant increase (p<0.05) in TAT and D-D levels was detected in bleeders with acute hematemesis and patients with PVT compared to cirrhotics with haemostatic balance. Moreover, FPS and PAI-1 levels were significantly elevated (p<0.05) in patients with PVT compared to those with acute hematemesis and were inversely correlated with platelet count (p<0.05). Other findings suggest that NE and TNF-α contribute to haemostatic alterations in patients with viral hepatitis C liver cirrhosis, and emphasize the clinical significance of protein C as a sensitive parameter for hepatic dysfunction and protein S and PAI-1 as reliable prethrombotic markers in these patients.

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SCREENING TEST FOR VON WILLEBRAND DISEASE IN CHILDREN: A PFA-100 CLOSURE TIME
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Von Willebrand disease (VWD) is the most common inherited bleeding disorder so clinical symptoms, positive family history and good sensitivity laboratory assays should be used for diagnosis. Definitive diagnosis of type 1 VWD remains a problem. There is a temporal variability in the level of von Willebrand factor (VWF) in association with stress, inflammation, drugs and pregnancy. Many patients with mild type 1 VWD sometimes have laboratory results in normal range and up to 40% of patients remain undiagnosed because of mild symptoms and borderline laboratory values. We evaluated the sensitivity of closure time (CT) of the PFA-100® system with both cartridges (collagen/epinephrine (EPI) and collagen/ADP (ADP)). Over a 5 year period (2000 - 2006) testing was performed on blood samples from 44 patients (age 0.3 - 19 years, median 12 years; 18 males and 26 females) registered in the Center for haemophilia and other bleeding disorders at the Children’s hospital in Medical centre Ljubljana, Slovenia. In house reference ranges for children population were previously established and are 78 - 160s for EPI test and 55 - 124s for ADP CT. We found that all 6 patients with type 2 or 3 VWD had prolonged CTs with both EPI and ADP cartridges. Among 38 patients with definite type 1 VWD 31 patients had prolonged CT with either of cartridges. The sensitivity of test for type 1 VWD was 96%, 100% and 88% of them (76%) had prolonged CT with EPI cartridge and 23 (59%) with ADP cartridge. On the day of CT testing 7 of 38 patients (18%) with type 1 VWD had results in normal range with both cartridges. Only 3 of these patients had low VWF level, other 4 (10%) had normal VWF level. Sensitivity of the PFA-100® system established in our patients with type 1, 2 and 3 VWD was 84%. When clinical suspicion is strong, testing for CT and VWF level should be repeated in spite of normal CTs and normal VWF level.

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ANGIOGENIC FACTORS PATTERN IN LYMPHOCYTIC LEUKEMIA
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Backgrounds. Angiogenesis is a crucial event in development and progression of solid tumors. Although the role of angiogenesis and angiogenic status is well studied in acute myeloid leukemia, its role in lymphocytic leukemia remains insufficiently characterized. Aim. is to investigate the profile of the systemic components of angiogenic factors in pediatric patients with acute lymphoblastic leukemia at diagnosis (n=28), in remission (n=14), and chronic lymphocytic leukemia at diagnosis (n=15), in remission (n=9) in order to determine their clinical validity. Methods. By ELISA technique, we assessed the serum vascular endothelial growth factor (VEGF), tumor necrosis factor-α (TNF-α), endothasin and basic fibroblast growth factor (bFGF) levels in culture supernatants of peripheral blood mononuclear cells collected from ALL and CLL patients at diagnosis and in remission. On the other hand serum matrix metalloproteinase-9 (MMP-9) was assayed in remission only. Results. In ALL patients, VEGF were significantly lower than control (p<0.001) and increased near control levels in remission (p>0.05). In contrast, bFGF level was significantly higher than that in control (p=0.045) and decreased near control level in remission (p>0.05). Both serum TNF-α and endothasin levels showed no significant difference both at diagnosis (p>0.05) and in remission (p>0.05) comparing to control level. Serum MMP-9 level was significantly lower than that in control (p=0.004). In CLL patients, serum VEGF, TNF-α and bFGF levels in culture supernatant were significant C than those in control (p=0.05). Conclusion. Our results suggest that serum levels of VEGF, bFGF and TNF-α are decreased in patients with chronic lymphocytic leukemia at diagnosis, however, at remission VEGF and TNF-α levels were still lower than controls. MMP-9 levels in patients with acute lymphoblastic leukemia at diagnosis and in remission were significantly lower than controls (p=0.009). Serum MMP-9 level at diagnosis was significantly higher than that in controls (p<0.05). Serum VEGF level at diagnosis was significantly lower than that in controls (p<0.05). Considering the role of Angiogenic factors in adult ALL, it appears different from CLL. Although the angiogenesis have a vital role in CLL its role in adult ALL is not so clear.

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ASSOCIATION OF PLASMINOGEN ACTIVATOR INHIBITOR-1 (PAI-1) GENE POLYMORPHISM AND CHANGES IN PAI-1 PLASMA CONCENTRATIONS WITH STROKE
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Background. Stroke is a major cause of morbidity and mortality, and rates as one of the leading causes of death and disability. As inhibitor of fibrinolysis, high levels of plasminogen activator inhibitor type 1 (PAI-1) reportedly increased the risk of cardiovascular disease, including stroke. Several factor influence PAI-1 levels, including the 4G/5G polymorphism of which the 4G allele is associated high plasma levels of PAI-1, and discordant results were reported on the association of the PAI-1 4G/5G polymorphism with stroke. Aims. Insofar as aberrant fibrinolysis was reportedly associated with heightened stroke risk, the aim of the study was to determine the allele, genotype, and haplotype distribution of the 4G/5G (rs1045519) PAI-1 polymorphism in stroke patients, and to assess the contribution of these genotype on PAI-1 and t-PA antigen levels. Methods. This was a case-control study performed on 173 patients aged 32-84 years with first ischemic stroke, confirmed
VASCULAR AND SINUSOIDAL ENDOTHELIAL ACTIVATION, PROLIFERATION, DIFFERENTIATION AND ERYTHROPHAGOCYTOSIS: ULTRASTRUCTURAL FINDINGS ON A CASE OF AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME (ALPS)

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Backgrounds. Since 1991, one of us (Sencer H) has reported that; vascular endothelial cells have reserved the capacity of stem cell and can activate, proliferate, and differentiate to other stromal and hematopoietic cells in health and diseases. Activated endothelial cells can migrate to the stroma or circulate in the vascular lumen as circulating progenitor endothelial cells (CEC/CPEC) after plamping and detaching from basal lamina. Besides, viral replications and damages on erythrocytes were clearly demonstrated ultrastructurally by Sencer H in 1995. Aims. The aim is to provide morphological basis of functional modifications occurring in the disease. This is the first ultrastructural study on ALPS, to our knowledge. Case Report and Methods. The patient was healthy until the age of 6 months when he presented with disseminated vesicular skin lesions, diagnosed as severe varicella zoster virus (VZV) infection. Coombs positive (IgG) hemolytic anemia, thrombocytopenia, elevated immunoglobulin levels and severe proteinuria were detected. CMV IgM and IgG were also found to be positive. At the age of 10 month CMV IgM and whole blood polimerase chain reactions analysis for CMV were negative. He presented with Evans syndrome symptoms and he was diagnosed as ALPS after the detection of increased percentage of double negative T cell population in the peripheral blood. The patient underwent splenectomy at the age of 20 months because of refractory thrombocytopenia. Material for this study was obtained during spleectomy and performed EM preparation. Semi-thin sections were stained with toluidine blue-borax. Thin sections were contrasted with uranyl acetate/lead citrate and observed with JEOL100BEM.

Results. Red pulp was widespread, but white pulp wasn’t distinctive with increased follicular hyperplasia and prominent marginal zone in the spleen. Increased fibrotic elements some of which related several arteries in plane of sections and plasmacytes were seen. Virus-like particles were observed. Activation and proliferation of vascular and sinusoidal (littoral) endothelial cells had occurred. Some of them were committed to erythropagocytosis which were became large and shuttle shape. Their cytoplasms were full with erythrocytes, erythrocyte fragments and/or phagocytic end-products. Erythrocytes probably damaged with viruses- were internalized by endothelial cells, but could not be digested totally. Both of the activated and phagocytic endothelial cells could detach from their original sites and move to the sinusoidal and/or vascular lumen. These could functionally be named circulating endothelial progenitor cells (CPEC) and circulating erythro-phagocytic endothelial cells (CEPEC) respectively. These were neither sinusoidal histiocytes, nor cordal macrophages classical. Conclusions. We suggest that the splenic endothelial cells have erythropagocyctic activity in ALPS. Viral replication on erythrocytes and/or endothelium may be causative agent. Endothelium should be most important key system in the health and disease. Electron microscopy is useful to avoid misinterpretation.

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ENDOTHELIAL MICROPARTICLES AND MARKERS OF COPPER METABOLISM AS NOVEL INDICATORS OF ANGIogenesis IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Backgrounds. Angiogenesis is currently considered an important process in biology of B-cell chronic lymphocytic leukemia (B-CLL). Copper is an important cofactor for some angiogenic factors. Elevated serum levels of copper (Cu) and its transport protein ceruloplasmin (CP) have been reported in patients with advanced cancers. Endothelial microparticles (EMPs) are fragments of endothelial cells which are produced during endothelial proliferation or damage and circulate in peripheral blood. Neither parameters of copper metabolism nor EMPs have been used so far to assess angiogenesis in B-CLL. Aims. To analyze serum concentrations of Cu and CP and quantitate EMPs in patients with B-CLL. Methods. We measured serum Cu and CP in 19 patients with B-CLL diagnosed according to NCI-WG criteria. Cu was measured using chromatography and CP by immunoturbidimetry. EMPs were analyzed in 20 B-CLL patients and 10 healthy donors using two-colour flow cytometry of platelet-poor plasma. CD105 (endoglin) and CD144 (VE-cadherin). CD41 was used as a platelet marker. Results. Cu and CP were detectable in all B-CLL patients. Both markers were in normal range (Cu: mean ± SD [standard deviation], 18.13±3.98 µmol/L, 95% Cl [confidence interval] of mean, 16.21-20.05 µmol/L; CP: mean±SD, 0.294±0.062 g/L, 95% CI of mean, 0.264-0.324 g/L). Neither Cu nor CP were significantly different between B-CLL patients with stable (n=7) and progressive (n=12) disease (p=0.77 and 0.54, respectively). There was a statistically significant increase of CD41+/105+ microparticles (mean ± SD 142.8 ± 22.4/µl, 95% CI of mean, 158.8-189.7/µl) in B-CLL patients when compared to control group (mean ± SD [standard deviation], 60.8 ± 32.4/µl, 95% CI of mean, 37.6-84.0/µl; p=0.008). There was no significant difference between patients with Conclusions. Our study is the first one to report measurement of endothelial microparticles and markers of copper metabolism as angiogenesis indicators in CLL. However, neither serum Cu nor CP were significantly elevated in B-CLL patients over controls. In addition, we did not observe differences in Cu or CP levels between patients with stable vs progressive disease. Furthermore, we found elevated numbers of CD41+/105+ (aggregates of platelets and EMPs) but not CD144+ or CD105+ EMPs in B-CLL patients. Larger study is clearly warranted to confirm these findings and perform a detailed statistical analysis including comparison with other angiogenic markers.

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ELIMINATION OF IRON IN HEREDITARY HEMOCROMATOSIS PATIENTS TREATED WITH ERYTHROCYTOPHERESIS
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Backgrounds. Hereditary hemochromatosis (HH) is an inherited, autosomal recessive disorder of iron metabolism that causes the body to absorb and store an excess amount of iron resulting in the progressive accumulation of iron in the liver, pancreas, heart, joints, and pituitary gland leading to potentially serious complications including cirrhosis of the liver, diabetes, and heart problems. The effective treatment is the regular whole blood removal which causes erythropoiesis activation and leads to decrease of iron stores. Red cell apheresis is an optional method for removing of higher amount of erythrocytes in one session. The aim of this study was to evaluate the effectiveness of erythropoiesis activation in the treatment of HH.

Methods. Repeated erythropoiesis activation were performed in 17 patients (with diagnosis of HH \(15/20\) haemolyzates, 2x C282Y + H63D heterozygotes) using Haemonetics MCS 3c cell separator (protocol TAE) in which red cells were removed from patients in 2 - 5 cycles; plasma and buffy-coat were reinused. Collection time, donor convenience, side effects and red cell yield were recorded and analyzed. Samples for hematology and iron studies in patients were drawn, analysed and compared to baseline levels. Results. 376 (3 - 70) red cell apheresis in 17 patients (15 male, 4 female), age 49.9 (32 - 67), height 175.7 cm (160 - 190), weight 82.8 kg (55 - 110), TBV 5186 ml (5627 - 6501). Procedure time was 32 - 87 min. Mean Hb level decreased from 141.7 g/L (115 - 185) before the procedure to 121.6 g/L (95 - 130). Ferritin values decreased from 1189 ng/ml (286 - 3996) to less than 25 ng/ml (7 - 23.9) in each of patients. The drop in ferritin level was 175 ng/ml (67 - 358) per month and 86 ng/ml (41 - 135) per one apheresis, respectively.

Conclusions. Procedures were well tolerated by patients, no serious side effects were seen, 21 mild citrate reactions (7.6%) were noted. Red cell apheresis is an effective procedure of iron stores reduction in patients with the hereditary hemochromatosis. Decrease of iron stores in patients is individual and depends on many factors.

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THERAPEUTIC LEUKAPHERESIS EXPERIENCE OF A SINGLE CENTRE
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Therapeutic Leukapheresis (TL) is an option in the management of patients with hyperleucocytosis, especially associated with leukostatic symptoms. Nevertheless, its clinical and analytical benefit is not well documented in the literature. The aim of this study was to retrospectively analyse the TL performed in our Centre, between January 1996 and December 2005 and also to evaluate its efficacy and complications. During this period, 28 TL were performed in 15 patients (9 mens/6 women), with a median age of 22 years (range 7-78), diagnosed with Acute Lymphoblastic Leukaemia (n=6), Acute Myeloblastic Leukaemia (n=7) and Chronic Myeloid Leukaemia (n=5) respectively. Most of the patients (n=14) initiated TL within one week after the diagnosis. One pediatric patient with CML and an initial white blood cells (WBC) count of 300 000 / L did not have leukostatic symptoms. The other presented cerebral (lethargy, aphasia, dysartha, altered vision, intracranial haemorrhage) and/or pulmonary (dry cough, respiratory distress and alveolar haemorrhage) manifestations. Each patient was treated with a median of 2 TL (1-4). Aphereses were performed in a Cobe Spectra cell separator in the Intensive Care Unit. The mononuclear cells program (MNC) was selected in 20 procedures and the polymononuclear cells program (PMN) in the other 8 cases. A median of 3 blood volumes per TL was processed (1-4). An efficacy index (EI) was calculated in order to monitor the procedures: EI = (total collected WBC / total pre-apheresis patient WBC) x 100. The median pre-apheresis WBC count was 213 000 / L (65-856), which had a corresponding median leukocrit of 8 ml/dl (2-26). The median EI of all TL was 20% (0-47) and when considering each program, the PMN had a median of 23% (16-47) and the MNC achieved 18% (3-30). The median WBC count, 1 hour and 24 hours after TL, was 17 400 / L (45-650) and 1 500 / L (0-941) respectively. Serious complications occurred in 4 patients leading to TL interruption. Those were: respiratory arrest, hypotension, respiratory failure and mucocutaneous haemorrhage; however no deaths occurred. Hypocalcaemia related side effects were observed in 13 patients, but promptly reverted with calci-
sively worldwide. It has been licensed since 1997 for use in the US and since 2005 in Europe. Aims. This post-registration, non-interventional study (EXELS: Evaluation of Xagrid Efficacy and Long-term Safety) was started on the initiative of the EMMA as a long term safety and efficacy study in a cohort of at-risk essential thrombocythaemia (ET) subjects exposed to anagrelide or other cytoreductive treatments. The primary study objective was to continuously monitor safety and pregnancy outcomes. Secondary objectives are to assess efficacy (platelet count and number of thrombohemorrhagic events) and drug utilization (including drug dose and duration of exposure). Methods. This 5 + 5 year European non-interventional study, which is being led by a steering committee of ET experts and continuously evaluated by an independent Data and Safety Monitoring Board, will enrol a minimum of 1000 at-risk ET subjects receiving anagrelide and up to 3000 at-risk ET subjects receiving other cytoreductive therapies. All cytoreductive agents must be prescribed in accordance with the appropriate product information. Subjects may be newly diagnosed or continuing their existing medication for the treatment of ET. Concomitant medication use is at the discretion of the investigator. Data will be collected without any interference with the treatment choice of physicians and will be captured electronically by use of a web-based registry utilizing electronic case report forms. An initial 5-year study period will focus on the collection of data related to a number of pre-defined events. These include complications of the disease (thromboembolic and hemorrhagic events as well as transformation) and possible toxic complications (congestive cardiac failure, cardiomyopathy, severe mucocutaneous disorders, pulmonary hypertension, pulmonary fibrosis/interstitial pneumonia, pancreatitis, rheumatoditis/myalgia and non-haematological malignancy). Death, as well as the incidence of serious adverse events related to current ET therapy will be recorded. Events will be evaluated by an independent Event Validation Panel. If required, based on review of data from the initial 5-year phase, data will be collected during a second 5-year study period to assess selected pre-defined events including pregnancy (and progeny) outcomes, serious adverse events related to current ET therapy, and other events as defined by the steering committee. Results. Study recruitment began on 30 May 2005. To date, 364 subjects have been recruited with the expected ratio of 1:2 between anagrelide and other platelet reducing therapy. Summary/Conclusions. This ongoing non-interventional study is expected to provide quality data from a large cohort of at-risk ET patients evaluating the long-term safety of agents used to reduce platelet levels in patients with ET as well as data regarding the protective power against disease complications.

1104 CHRONIC NEUTROPHILIC LEUKAEMIA WITH AN ASSOCIATED V617F JAK2 MUTATION: EVIDENCE OF MONOCLONAL ORIGIN OF T LYMPHOCYTE AND GRANULOCYTE LINEAGES

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Backgrounds. Chronic Neutrophilic Leukaemia (CNL) belongs to the atypical myeloproliferative group of disorders (MPD) and is a rare disease entity. It is characterised by a short term survival and transformation to acute leukaemia. Previously, we have described an individual with CNL which has survived more than 108 months with the disease. Subsequently, the patient has exhibited evidence of myelofibrosis and transformation to a refractory anaemia with excess blasts (RAEB) 2 with clonal evolution as indicated by the recent detection of a cell population with chromosome 7 monosity. Screening for the V617F Janus Kinase (JAK) 2 mutation, which is highly prevalent in classical MPDs and in particular polycythaemia vera, the patient proved to be homozygous for the mutation in the granulocyte and macrophage lineages (McLornan et al 2005) Haematologica 90:1696. There is evidence to suggest that in the case of CNL both granulocytes and T cells can be derived from the same clone [Bohm et al (2003) J Clin Pathol 56:292]. Consequently, if the granulocyte lineage was positive for the V617F JAK2 mutation then the T lymphocytes would also be positive. Aims. To investigate whether the T and B cells are positive for the V617F JAK2 mutation and derived from the same clone as the granulocytes in a patient with advancing CNL. Methods. B lymphocytes were isolated by CD19+ immunomagnetic selection from the total lymphocyte fraction prepared from density gradient Ficoll separated whole blood. The remaining cells post immunomagnetic selection, which were T lymphocytes and natural killer (NK) cells, were retained. DNA was prepared from both B and T lymphocyte cell fractions and PCR-direct sequencing was performed. Results. Screening for the V617F mutation by sequencing indicated the B lymphocytes exhibited wild type JAK2 gene. Conversely, sequencing the cellular fraction containing isolated T lymphocytes and NK cells indicated clearly they displayed heterozygosity for the V617F JAK2 mutation. Summary. Screening for the V617F JAK2 mutation demonstrated the mutation was absent in the B lymphocytes but was present in the T lymphocyte lineage. Previously, we detected this same mutation in the homozygous state in granulocyte and macrophage lineages but it was absent in cells derived from the bursac cavity and hair follicles, thus confirming that the mutation was acquired. Since the T lymphocytes and granulocytes were positive for the V617F JAK2 mutation it would suggest that both lineages were derived from the same neoplastic clone in this case of CNL. Thus our results confirm the previous observations reported by Bohm et al (2005) in 4 CNL patients using Humara assays, which indicated monoclonality of T and granulocyte lineages. Finally, it remains to be established what exact role the V617F JAK2 mutation, which gives cells a proliferative advantage, plays in the pathogenesis and prognosis of rare atypical MPDs such as CNL.

1103 THROMBOTIC AND HEMORRHAGIC EVENTS IN PATIENTS WITH ESSENTIAL THROMBOCYTHAEMIA (ET) DURING THERAPY WITH INTERFERON-α OR ANAGRELIDE


Backgrounds. Essential thrombocythaemia (ET) is a clonal myeloproliferative disease characterised by sustained thrombocytosis and increased number of megakaryocytes in the bone marrow. The most severe complications and the principal causes of death in these patients include thrombosis, haemorrhage and progression to myelofibrosis, or acute myelogenous leukemia. Several agents have been reported to control the disease. Two of the most frequently administered are interferon and anagrelide. Aims. To retrospectively evaluate the incidence of thrombotic or hemorrhagic events in patients with ET during therapy with interferon-α or anagrelide. Methods. In a cohort of 195 patients with ET, who are followed up in our center during the last twenty years, we recorded the number of thrombohemorrhagic events as well as transformation and other adverse events related to current ET therapy, and other events as defined by the steering committee. Results. Study recruitment began on 30 May 2005. To date, 364 subjects have been recruited with the expected ratio of 1:2 between anagrelide and other platelet reducing therapy. Summary/Conclusions. This ongoing non-interventional study is expected to provide quality data from a large cohort of at risk ET patients evaluating the long-term safety of agents used to reduce platelet levels in patients with ET as well as data regarding the protective power against disease complications.
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**SYSTEMIC MASTOCYTOSIS: AN ITALIAN MULTICENTRIC RETROSPECTIVE SURVEY**


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Background/Aims. To evaluate clinical and molecular features, and outcome of patients (pts) with Systemic Mastocytosis (SM). Methods: A retrospective revision of 26 cases of SM, diagnosed in 11 Italian Hematology Divisions between 1995 and 2006. Results: 26 new cases of SM were collected and classified according to the WHO criteria: Mast Cell Leukemia in 12 pts, Aggressive SM in 10 and Indolent SM in 3; the remaining one had SM with associated clonal non-mast-cell-lineage hematologic disease (AML). Skin was the principal extramedullary organ involved by uncontrolled proliferations of MC (17 pts) followed by spleen (15), liver (12), and cardiovascular system (12). In 61% of cases constitutional symptoms (fatigue, itchness and abdominal pain) were present. Molecular biology studies were performed in 16 pts. 12 showed the c-kit point mutation D816V, in 3 pts additional gene defects and karyotype abnormalities were recognized. Treatments were very heterogeneous, and the same patient could have received different therapies after failure of the previous one. Seven patients were not initially treated: 5 maintained a stable disease, while 2 had a progressive clinical course. IM (400 mg/day) was used in 15 pts (10 as first line therapy, 4 and 1 as second and third line respectively); c-kit mutation was present in 9 of these 15 pts. A partial response was obtained in only one of them (response rate 11%); among the remaining 6 patients without the c-kit mutation, partial or complete remissions were obtained in 2 and 1 pts respectively (33% and 17%). Interferon-α (5×3 million units s.c. weekly) was employed in 6 patients (3 as first line therapy, 2 as second and 1 as third line); a partial remission was achieved in one case only (17%). 2 CDA (0.14 mg/kg) was administered in 3 pts (1 as first, 1 as second and 1 as third line therapy) registering a partial remission in all of them. One patient performed only radiotherapy and achieved a pure clinical major response. In 4 cases were used other chemotherapies (in 2 pts as first and in other 2 as second therapy) with no response, and 3 received steroid therapy, not in association with other drugs, obtaining in 1 case a partial response. Two patients underwent stem cell transplantation as second and fourth line respectively, obtaining both a complete remission. Two pts (8%) who had received conventional chemotherapy only, died for mastocytosis; a third patient in complete remission of disease died for accidental causes. The 10-years survival rate is about 88%. Summary/Conclusions. Our results suggest that SM is a very rare disease, but although severe and life-threatening mediator-related symptoms, the mortality is low. D816V c-kit mutation is associated with relative resistance against Imatinib. Among purine analogues, 2-CDA has shown interesting clinical response, while INF has not offered any benefits although the similarity between SM and myeloproliferative diseases. Because of the rarity of this disease, an effective standard of care is lacking: for this reason more data are needed to find new and successful therapeutic strategies, such as other tyrosine kinase inhibitors.

**1106**

**COMPARISON OF THE RESULTS FOR THE JAK2 V617F MUTATION DETECTION BY TWO METHOD: ALLELE SPECIFIC PCR AND RESTRICTION DIGESTION ASSAY AND POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTOSIS DNA SAMPLES**

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JAK2 V617F is a clonal acquired mutation found in the majority of patients with polycythemia vera (PV), and a significant number of patients with essential thrombocythemia (ET) and chronic idiopathic myelofibrosis (CIMF). The incidence of this mutation ranging from 65% to 97% in PRV patients, from 25% to 57% in ET patients and about 50% in CIMF depending on the study. These variations in percentage of patients involved is likely due to the criteria used for diagnosis and also the sensitivity of the assay used to detect this mutation. Allele specific PCR and restriction digestion assay by enzyme BsaXI are used techniques detection. This aim of this study was to compare for the first time results for the JAK2 V617F mutation detection by two frequently used techniques on DNA of granulocytes of the PV and ET patients. Furthermore, the results were compared to that published in the literature. The diagnosis of ET and PV was established followed World Health Organization classification. 19 patients with PRV and 42 patients with ET were included in the study. EDTA peripheral blood was drawn and used for the isolation of the granulocytes by Ficoll density centrifugation followed by dextran sedimentation. DNA was isolated from granulocytes by High Pure PCR Template Reagent kit from Roche The allele specific PCR was carried out as described in Baxter EJ et al. (Lancet 2005; 365:1054-61). Restriction digestion assay by enzyme BsaXI was carried out by test, designed by InvivoScribe Technologies (San Diego, USA). The PCR and restriction digestion products were visualized after agarose gel electrophoresis by ethidium bromide staining. The concordance between these two methods was 100%. The percentage of positivity for JAK2 V617F mutation on DNA samples from granulocytes from peripheral blood in ET and PV patients was similar of that published in the literature and was in the upper part of the range.

**1107**

**MICROVESSELS DENSITY (MVD) AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IMMUNOHISTOCHEMICAL EXPRESSION IN PH(-) CHRONIC MYELOPROLIFERATIVE DISORDERS (CMPDS)**


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There is increasing evidence that neovascularization may play an important role in haematological malignancies and in particular in lymphomas, acute leukemias and myelodysplastic syndromes. However, few studies have been performed in order to evaluate this phenomenon in Ph(-) CMPDs. Increased angiogenesis in chronic myeloproliferative disorders (CMPD) and high serum concentration levels of VEGF, the most potent direct-acting angiogenic factor, in CMPDs were reported. Recently, an increased immunohistochemical expression of VEGF was demonstrated in CMPDs. A new classification of chronic CMPDs was worked out by the WHO, which highlighted the importance of bone marrow biopsy (8MB) in differential diagnosis and in the evaluation of myelofibrosis. In addition to standard therapy, new therapeutic ways of approaching and directly targeting endothelial cells or VEGF have been experimented in CMPDs, with variable results. The aim of this research was to examine the MVD and VEGF immunohistochemical expression in the different categories of Ph(-) CMPDs, approached to the new WHO classification. We examined the BMBs of 90 CMPDs patients, classified according to the WHO classification. In particular, there were 30 cases of essential thrombocythaemia (ET), 30 of CML (10 CML-0, 10 CML-1 and 10 CML-2+3 sec CCGM - Haematologica 2005) and 30 of polycythaemia vera (PV) (20 polycythaemic phase and 10 polycythaemic myelofibrosis); we analyzed 30 non-pathologic BMBs as normal controls. MVD analysis was performed according to the hot-spot methods, using an anti-CD34 antibody. The VEGF immunohistochemical expression was expressed as VEGF index, according to the mathematical formula [VEGF(i) = VEGF(+) x BMB cellularity/100]. All statistical tests were performed at the 5% significance level (p<0.05) (Anova one way). Hot-spot MVD and VEGF(i) immunohistochemical results are described in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Hot-spot MVD and VEGF(i) immunohistochemical results.</th>
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<tbody>
<tr>
<td><strong>Vessels Medium</strong> N. ± SD (range) <strong>VEGF(i) ± SD (range)</strong></td>
</tr>
<tr>
<td>Controls</td>
</tr>
<tr>
<td>ET</td>
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<tr>
<td>CIMF-0</td>
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<tr>
<td>CIMF-1</td>
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<tr>
<td>CIMF 2+3</td>
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<tr>
<td>PV</td>
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<tr>
<td>MF post-PV</td>
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There is no difference in MVD and VEGF(i) expression between ET and control group. Moreover MDV and VEGF(i) proved to be much
higher in CIMF and PV than in the control group. MVD and VEGF(i) in fibrotic CIMF (CIMF-2-3) have been demonstrated statistically different from MDV and VEGF in myelofiobrosis post-PV. Our analysis identified significant biological differences between the various types of myelofiobrosis and could serve as a rationale guide in the antiangiogenic therapy.

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JAK2V617F, PRV-1 EXPRESSION AND ENDOGENOUS ERYTHROID COLONIES GROWTH IN PATIENTS WITH POLYCYTHEMIA VERA


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Background. Polycythemia vera (PV) is a chronic myeloproliferative disorder (MPD) characterized by a primary increase of red cell mass. One of the WHO diagnostic criteria for PV is the in vitro endogenous erythroid colonies (EEC) formation. Some molecular alterations have been associated to MPD, and currently the most indicative molecular markers are the overexpression of PRV-1 and the genomic mutation JAK2V617F. Both these alterations have been found in the majority of PV cases and in particular JAK2 has been associated with the ability to form EECs, and seems to have a causal or a strongly contributory role in the pathogenesis of the MPDs and in particular of this primary erythrocytosis. Furthermore it has been suggested an allele dose-dependent association that links JAK2V617F and expression of PRV-1. Aims. To evaluate the association between EECs formation and the molecular alterations considered, we analyzed 21 cases of PV for EECs, JAK2V617F and PRV-1 expression. Methods. JAK2V617F was performed by allele-specific amplification, PRV-1 expression was normalized on GAPDH expression, analyzed with the ΔCt method and expressed as relative quantification (RQ); EECs were detected on methylcellulose-based medium with and without erythropoietin addition. Results. JAK2V617F was found in 17/21 (81%), and 4 of the JAK2V617F-negative cases presented only the mutated allele. All the patients analyzed showed EEC growth. PRV-1 expression was evaluated in 14 patients at the diagnosis and in 7 patients under hydroxyurea administration; overexpression resulted in 15/15 and 3/7 (43%) patients, respectively. The RQ mean in the JAK2V617F-negative, heterozygous JAK2V617F-positive and homozygous JAK2V617F-positive groups of untreated patients resulted 3.7 (1.2-10.4), 10.95 (3.6-25) and 12 (1.2-24), respectively. A group of 7 patients with secondary erythrocytosis was also analyzed and resulted JAK2V617F-positive did not display higher values than JAK2V617F-negative patients. The RQ mean in the JAK2V617F-negative, heterozygous JAK2V617F-positive and homozygous JAK2V617F-positive groups of untreated patients resulted 3.7 (1.2-10.4), 10.95 (3.6-25) and 12 (1.2-24), respectively. A group of 7 patients with secondary erythrocytosis was also analyzed and resulted JAK2V617F-positive did not display higher values than JAK2V617F-negative patients. The RQ mean in the JAK2V617F-negative, heterozygous JAK2V617F-positive and homozygous JAK2V617F-positive groups of untreated patients resulted 3.7 (1.2-10.4), 10.95 (3.6-25) and 12 (1.2-24), respectively. A group of 7 patients with secondary erythrocytosis was also analyzed and resulted JAK2V617F-positive did not display higher values than JAK2V617F-negative patients.

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JAK2V617F MUTATION, PRV-1 OVEREXPRESSION AND EECs IN PATIENTS WITH ESSENTIAL THROMBOCYTHERIA

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Background. JAK2V617F is a genomic mutation associated to myeloproliferative disorders such as polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis, that has been highly correlated with the ability to form endogenous erythroid colonies (EEC) and with PRV-1 overexpression. Nevertheless JAK2V617F mutation and PRV-1 overexpression are present in the majority of PV but in a percentage significantly lower of patients with ET, suggesting a different role or effect of these molecular alterations in the different chronic myeloproliferative disorders. In particular the presence of JAK2V617F mutation is associated in ET patients to multiple clinical features resembling PV, suggesting the need of a new diagnostic classification based on the genotypic profiles. Aims. In the attempt to evaluate the incidence of the genetic alterations described, and the association to hematological features, we analyzed JAK2V617F, PRV-1 expression and EEC growth in a cohort of 46 patients with essential thrombocytemia. Methods. JAK2V617F was performed by allele-specific amplification, PRV-1 expression was normalized on GAPDH expression and analyzed with the ΔCt method, the EECs were detected on methylcellulose-based medium with and without erythropoietin. Results. JAK2V617F mutation was present in 11/46 (24%) cases; PRV-1 was overexpressed in 12/43 (27.9%) cases, in particular 7/27 untreated patients (25.9%) and 5/16 (31.3%) patients in therapy; 30/39 (76.9%) cases showed the ability to form EEC. The statistical evaluation of the data showed a significant correlation between JAK2V617F and EEC growth (p=0.04, R=0.33), but the correlation between JAK2V617F and PRV-1 overexpression was not significant. Conclusions. Our study seems to be in accordance with previous reports regarding the incidence of the molecular alterations found, but the correlation was statistically significant only between JAK2V617F and EEC. In fact, considering PRV-1 overexpression, neither correlation was statistically significant, nor was found any allele dose depending effect on PRV-1 expression by JAK2V617F mutation. Finally, we did not find a difference between cases analyzed at the diagnosis and cases that were in therapy. Considering hematological parameters such as white blood cells and platelet counts, and hemoglobin level, the subgroup JAK2V617F-positive did not display higher values than JAK2V617F-negative patients. Thus we did not find distinctive hematologic characteristics that differentiate ET JAK2V617F-positive or negative.

1110

INCREASED ANGIOGENESIS IN CHRONIC IDIOPATHIC MYELOFIBROSIS: VEGF AS KEY ANGIogenic FACTOR

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Background. Recent studies suggest that increased angiogenesis is implicated in the pathogenesis of chronic idiopathic myelofibrosis (CIMF). Its impact on prognosis, however, is still a matter of debate. Vascular Endothelial Growth Factor (VEGF) is a potent stimulator of angiogenesis which is expressed in virtually all types of malignancies. We therefore hypothesised that VEGF may also play a role as an angiogenic mediator in CIMF Aims. The purpose of this study was to assess the prognostic value of bone marrow angiogenesis and its correlation with clinical parameters and cytogenetics in patients with newly diagnosed, untreated CIMF. Moreover, we aimed to investigate the expression of VEGF in bone marrow of CIMF patients. Patients and Methods. All 102 CIMF patients were enrolled in the study. Between 1990 and 2001 all patients who were diagnosed as having CIMF at our center and for whom adequate bone marrow sections and clinical data were available, were deemed eligible. Each case was re-classified according to WHO-criteria. As a surrogate marker for angiogenesis we used microvesSEL density (MVD) as assessed by CD34 staining on paraffin-embedded trephine biopsy specimens. VEGF expression was examined by standard
immunohistochemical technique. The cytogenetic phenotype was determined by FISH, de-paraffinized bone-marrow sections. Appropriate summary statistics were used for comparisons between groups; survival was calculated using Kaplan-Meier estimators. Parameters found to be of prognostic significance in univariate analysis were verified in a multivariate Cox regression model. Results. Fifty-five patients were included in this retrospective single-center study. Clinical, cytogenetic and immunohistochemical data were available for all patients. With a median follow-up of 52.4 months (range 1 - 142 months), the median overall survival of the study cohort was 76.8 months. With a median MVD of 45 per 0.747 mm² field (range 6-96) CIMF patients displayed a significantly higher degree of bone marrow microvascel density than age-matched controls (n=10; median MVD=19, range 4-23; p<0.001). In fact, 85% of CIMF patients displayed an elevated MVD compared to normal controls. MVD was elevated significantly at all CIMF stages (p=0.001) with equal distribution between the various degrees of fibrosis (MF 0 - 5). Accordingly, VEGF expression was significantly higher in CIMF (median 12 cells per 0.747 mm² field) compared to normal controls (median 1.4 cells per 0.747 mm² field; p=0.01) and correlated with MVD (p=0.001). However, we found no correlations of MVD or VEGF expression with cytogenetics and clinical outcome, respectively. Conclusions. Our study confirms that bone marrow angiogenesis is increased in CIMF. In parallel, we found significantly elevated VEGF expression suggesting VEGF signaling plays a pathogenetic role and representing a potential therapeutic target in CIMF.

**1111**

**JAK2-V617F MUTATIONAL ANALYSIS IN PATIENTS WITH MYELOPROLIFERATIVE DISORDERS**

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Backgrounds. An acquired mutation in Janus kinase 2 (JAK2) gene (characterized by a valine-to-phenylalanine substitution at position 617 [V617F] in the JH2 domain) has been recently described in the majority of patients with myeloproliferative disorders (MPDs). This mutation is associated with constitutive phosphorylation of JAK2 and its downstream effectors as well as induction of erythropoietin hypersensitivity in cell lines. However, its precise role remains to be determined. The aim of this study was to estimate the prevalence of the JAK2-V617F mutation, as well as the clinical and laboratory findings in patients with MPDs carrying the mutation. Materials and Methods. One-hundred and forty-two patients (M/F: 72/70, mean age: 59.8 years, range: 24-88) suffered from essential thrombocythemia (ET, 94), polycythemia vera (PV, 59) and idiopathic myelofibrosis (IF, 9) were selected retrospectively from outpatient clinic between January 2000 and February 2006. Forty-five patients (51.7%) had at least one confirmed arterial and/or venous thrombotic episode either at diagnosis (mainly, as well 1-2 years ago) or during the follow-up period (mean: 38.8 months, range: 2-171). Genomic DNA was extracted from bone marrow aspirates or peripheral blood using standard protocol. The JAK2-V617F mutation was detected using both allele specific PCR, and PCR-RFLP assay. Variables analyzed included age, gender, survival, thrombotic events, WBC, Ht, Hb, PLT, Epo, LDH, and the presence of splenomegaly, hepatomegaly and antidiinopin antibodies both at diagnosis and during the follow-up period. Statistical analysis was performed by the SPSS software. Results. One-hundred and three patients harbored the JAK2-V617F mutational activity (53 of 39 with PV, 84.7%; 63 of 94 with ET, 67.02%; 7 of 9 with IF). Interestingly, the patients carrying the JAK2-V617F mutation were older at diagnosis (61.3 vs 55.5, p=0.026), displayed lower Epo levels (8 vs 19.6, p=0.001), higher Ht (45.6 vs 42.1, p=0.018) and Hb values (15.1 vs 15.3, p=0.021), and presented more often with thrombotic events (35.9% vs 20.5%, p=0.079) and splenomegaly (42.65% vs 17.9%, p=0.007). In multivariate regression analysis, Epo levels and the presence of thrombotic events were independent variables correlated with the presence of mutation (p=0.004 and p=0.038, respectively). Moreover, 4 out of 5 patients who exhibited progression of the disease (5 with ET to IF and two with PV to IF and AML, respectively) displayed the mutation, both before and after the deterioration of the disease. Conclusion. MPDs with JAK2-V617F mutation may prove to be a different disease entity than MPDs without JAK2-V617F mutation, with distinct clinical and laboratory findings.

**1112**

**ROSAI-DORFMAN DISEASE (SHML) WITH NODAL AND MULTIPLE EXTRANODAL INVOLVEMENT COMPLICATED WITH AUTOIMMUNE HEMOLYTIC ANEMIA - A CASE REPORT**

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Background. Rosai-Dorfman disease or Sinus Histiocytosis with Massive Lymphadenopathy (SHML) described by Rosai and Dorfman in 1969, is a rare disorder (423 cases in the SHML registry) of unknown etiology, characterized by a nonmalignant proliferation of distinctive histiocytic/phagocytic cells within lymph node sinuses and lymphatics in extranodal sites (50%-60% of cases). Aims. Presentation of a patient with a severe form of SHML with nodal and multiple extranodal involvement (skin, upper respiratory tract, parotid gland, thymus), complicated with autoimmune hemolytic anemia. Methods. A gipsy male patient, one year old, was admitted with high fever, night sweats, asthenia, and loss of weight, inspiratory stridor, massive painless cervical and submandibular lymphadenopathy, moderate hepatomegaly and splenomegaly. Extensive investigations were performed: imagistic, hematological, biochemical, immunological, serological, bacteriological, histopathological. Results. On examination, the patient was pale, moderately undernourished, had pronounced inspiratory dyspnea with stridor and dysphonia; the cervical, and submandibular lymph nodes were grossly enlarged bilaterally. With a cobblestone consistency, nontender. The parotid glands were enlarged. The x-Ray and CT scan also showed mediastinal and hilar lymphadenopathy and thymus enlargement. In evolution the patient developed a frank suprerior mediastinal syndrome, the axillary, inguinal and retroperitoneal lymph nodes were involved, and the hepatosplenomegaly progressed. The skin lesions located on the eyelids and periorbitar were nodules, and those on the thorax and limbs, pruritic erythematous papules. Biological investigations: moderate anemia, complicated than with Coombs positive anemia necessitating repeated transfusions, leukocytosis with neutrophil predominance, bone marrow with granulocytic hyperplasia; elevated ESR, high levels of γ globulins, IgG, fibrinogen, triglycerides, and ferritin; markedly decreased number of CD4+ cells and CD4+/CD8+ ratio, normal number of NK cells; serologic markers for EBV, HHV, CMV, HIV were negative; rheumatoid factor and antinuclear antibodies negative. Repeated lymph nodes and skin lesions biopsies were performed. The histopathological investigation failed to recognize SHML, giving different interpretations (Hodgkin disease, non-Hodgkin lymphoma, reactive lymphadenopathy); finally, the recognition of sinus histiocytosis and the hallmark of the SHML histiocyte, the lymphphogacytosis (emperipolesis), completed with immunohistologic investigation (histiocytes CD68+, S 100 protein+, CD1a-) confirmed the diagnosis. The treatment with prednison 60 mg/m² for two months was efficient but the clinical and biological symptoms relapsed one month after. The interferon treatment was totally inefficient. Taking into account the severity of the disease, the treatment was continued with dexamethasone, etosopide and cyclosporine, for 52 weeks (HLH-94 protocol). The response was very good, with complete recovery maintained 32 months after completing the treatment. Some peculiar features of the case are interesting: common manifestations with the hemophagocytic lymphohistiocytosis (HLH)-important hepatosplenomegaly; high triglycerides and ferritin levels, entrophagocytosis; the autoimmune hemolytic anemia - recently case of SHML associated with autoimmune lymphoproliferative syndrome (ALPS) have been described. The possibility that SHML represents an acquired disorder of apoptosis has raised a special interest. Conclusions. Being an extremely rare disease, SHML was recognized very late, despite its characteristic histopathologic features. The particular severity of the presented case, with extensive involvement, progressive evolution and life threatening complications (mediastinal syndrome, hemolytic anemia), imposed an intensive treatment.
S. Kaviani, × 3 × × and informative peaks M. Bengochea, Switching of fetal hemoglobin (Hb F) in the adults was ()). DNA was obtained A.I. Alvarez software. Depending on the locus, Congress of the European Hematology Association E. Carreto, Medical Sc, Timisoara, Iran cells. The hematopoietic colony forming assay showed that hematopoietic sion detected by semiquantitative RT-PCR in comparison with control. 2 patients (21 to 14 days, and 16 to 12 days, respectively) when palifer- to 8 days, respectively) and the duration of hospital stay was reduced in mucositis of grade 3, 2, or 1 in severity was recorded. Pyrosis and abdom- tis in all 3 patients, in comparison to the first PBSCT where WHO oral mucositis were compared between the first and second PBSCT orography. These results were confirmed by increase of γ globin expression detected by semiquantitative RT-PCR in comparison with control. The hematopoietic colony forming assay showed that hematopoietic progenitor cells have ability to forming colony the same as untreated cells. Summary/Conclusions. In conclusion, the cytokines or its derivatives that are used in this study can be a suitable candidate for treatment and investigation purposes instead of conventional drugs that can increase the Hb F.

1114 HIGH-DOSE MELPHALAN WITH OR WITHOUT PALIFERMIN IN MULTIPLE MYELOMA: A SELF-CASE-CONTROL STUDY

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Oral, oesophageal, and lower gastrointestinal tract mucositis is a common complication of high-dose chemotherapy conditioning regimens used with peripheral blood stem-cell transplantations (PBSCT). Severe grades of mucositis are associated with higher morbidity such as infections, need for parenteral nutrition, opioid analgesics and prolonged hospitalization. Moreover, oral mucositis is reported by patients as the worst and most memorable complication of their transplant experience. The KGF (keratinocyte growth factor) palifermin stimulates the growth, differentiation, migration and survival of epithelial cells. Palifermin is now approved in the EU to decrease the incidence, duration and severity of oral mucositis in patients with haematologic malignancies requiring autologous hematologic stem-cell transplantation. Prior to marketing approval, Palifermin was made available to patients in the UK through an early access program. This report describes the treatment of 3 patients who received drug through this program. To evaluate the effect of Palifermin on oral mucositis given during the second autologous transplant in 3 patients who underwent a double PBSCT for multiple myeloma. Three multiple myeloma patients were scheduled to receive treatment with high-dose (HD) Melphalan (200 mg/m²) followed by two successive autologous PBSCT. At the time of second autograft, patients received prophylactic intervention with intravenous Palifermin 60 μg/kg/day for 3 days before HD Melphalan and for 5 days after PBSCT. Mucositis prevention, hematologic growth factors, parenteral nutrition and all other supportive care were identical during the two PBSCT and followed institutional protocol. Regimen-related toxicity, particularly mucosal toxicity were compared between the first and second PBSCT with each patient representing its own control. Oral mucositis was assessed according to the WHO oral-toxicity scale. 4. Results, Palifermin use during the second PBSCT prevented the occurrence of oral mucositis in all 3 patients, in comparison to the first PBSCT where WHO oral mucositis of grade 3, or 1 in severity was recorded. Pyrosis and abdominal pain lessened in severity in 2 of 3 patients, but the severity of diarrhea did not change (2 patients) or was worse (1 patient). The duration of parenteral nutrition was reduced in 2 patients (15 to 14 days and 13 to 8 days, respectively) and the duration of hospital stay was reduced in 2 patients (21 to 14 days, and 16 to 12 days, respectively) when palifermin was administered with the conditioning regimen. Side-effects of Palifermin were noted in all patients and consisted of pruritis (1), erythe- ma (1), mouth and tongue disorders (3) and edema (1), but were all mild to moderate in severity and self-limiting. 5. Conclusions. In comparison to the first autologous PBSCT after high-dose melphalan where oral mucositis of grade 3, 2 and 1 in severity was recorded without intervention, Palifermin use prevented the occurrence of oral mucositis in all 3 multiple myeloma patients, and lessened the clinical signs of oesophagi- tis and diarrhea in 2 of the 3 patients undergoing double PBSCT. Further evaluation in larger but similar populations would be of interest to confirm these encouraging results.

Table 1. Characteristics of patients and side effects after double transplantation procedure.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Side effects</th>
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<tr>
<td>Patient 1</td>
<td>Myeloma IgG III</td>
<td>Oral, oesophageal, and lower gastrointestinal tract mucositis</td>
</tr>
<tr>
<td>Patient 2</td>
<td>Myeloma IgG III</td>
<td>WHO Grade Oral Mucositis</td>
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<tr>
<td>Patient 3</td>
<td>Non-secretant Myeloma III</td>
<td>WHO abdominal pain, colitis</td>
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1115 LINEAGE SPECIFIC CHIMERISM ANALYSIS ALLOWS EARLY DETECTION OF RELAPSES AFTER ALLOGENIC STEM CELL TRANSPLANTATION

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Backgrounds. Chimerism analysis is essential to verify the origin of hematopoiesis after allogeneic stem cell transplantation (SCT). Considering that after SCT, almost all relapses are recipient-derived, the reappearance of mixed chimerism or an increasing fraction of recipient-derived cells should prompt the suspicion of relapse and be differentiated from graft failure or rejection. Furthermore, as reduced intensity conditioning SCT (RIC-SCT) emerge as a frequent procedure, a correct interpretation of chimerism analysis becomes imperative since transient mixed chimerism is frequently observed after RIC-SCT and does not necessarily means an unwanted evolution. Aims. To evaluate the usefulness of our methodology of lineage-specific chimerism analysis to sensitively detect relapse early after conventional or RIC SCT. Methods. We performed chimerism analysis in whole peripheral blood (PB) as well as in the separated cells on days 14, at the time of neutrophil recovery and monthly thereafter during the first year after SCT. Chimerism was determined on PB by short tandem repeat (STR) analysis on unfractonated PB or after cell separation (lineage-specific chimerism: positive selection of mononuclear cells using CD3, CD19 and CD19 monoclonal antibodies conjugated with magnetic beads; Dynabeads®). DNA was obtained with the Miller method and samples were used in a multiplex polymerase chain reaction to amplify 6 (D8S1130, D21S1270, D6S1031, D22S685, D11S1392, D3S2398, D5S2501, D15S657, D10S1237, IFNAR-1) STRS loci. Primers were marked with Cy5. Separation and detection of fragments were done with ALF-Express® and informative peaks were analyzed with the AlleleLinks® software. Depending on the locus, sensitivity to detect mixed chimerism was evaluated in 1 to 5%. Results. Fifteen patients were allografted at Maciel Hospital, Montevideo, Uruguay, from January 2005 to December 2004 and those with at least
I chimerism analysis were included (n=13). Five patients relapsed during the first year after SCT. Three of them were detected by chimerism analysis: in one case, mixed chimerism was observed in the subpopulation compromised by the disease (CD19+ in B lineage ALL with CD19 positive blasts) while in the other two patients, relapses were detected by an increasing recipient hematopoiesis in unfractinated blood and CD3-CD19-CD15 subpopulations (AML with CD15+, CD8- and CD19-blasts and ALL with CD19+, CD8- and CD15-blasts). The other two patients had relapses of CML that were detected by nested PCR for bcr/abl and cytogenetic analysis but did not show mixed chimerism. Conclusions. These results suggest that, at least in some diseases, line-age specific chimerism could be an alternative to other methods to increase sensitivity and specificity of relapse detection.

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EXPRESSIOn OF TGF

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Backgrounds. Tissue Growth Factor β is a multifaceted cytokine involved in several important life processes including hemostasis, tissue growth and immune suppression. It is active at pM concentrations and its actions are mediated via cell surface heterotrimeric receptor (TGF-β1, -β2, -β3) complexes. Recently, several groups have identified TGFβ on the surface of the immunoregulatory CD4+CD25+ (Treg) cells in mice and man, and certain groups have provided evidence for the existence of regulatory T-cells bearing this cytokine on their surface. Aims. We have established that the majority of Treg cells in the peripheral blood of normal human donors express TGFβ on their surface, and wished to explore further the possibility that other types of peripheral blood cells might do so as well. Methods. Peripheral blood from healthy normal donors (n=16), 5 M (20-45) years, were studied. They were studied at diagnosis and after 3 courses of chemotherapy. The control group consisted of 15 age-matched normal donors. The presence of CD158b and CD94:NKG2A was evaluated in NK cells and CIK, isolated from patients and normal donors. Peripheral blood mononuclear cells (PBMCs) were obtained by density-gradient centrifugation (Ficoll-Hypaque) of heparinized venous blood.

Table 1.

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<th>NK cells</th>
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<td>CD158b</td>
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These were then counted microscopically. Three colour immunofluorescence staining was performed. Seven ml of monoclonal antibodies conjugated with FITC, PE and Cy-Chrome was added to each tube. We used the following monoclonal antibodies specific for: CD14, CD45 (DAKO, Denmark), CD3, CD56, CD94, CD158b and NKG2A (Becton Dickinson, USA). Tubes were then agitated and incubated for 20 min. at 4°C, before which 5 ml of PBS-Ca2+/Mg2+ containing 0.1% NaN3 (Sigma Chemical Co., St Louis, Mo, USA) was added to each tube and pelleted by centrifugation (1200 rpm for 10 min). The supernatant was removed and the pellet resuspended in 0.2 ml PBS-Ca2+/Mg2+ with 0.1% NaN3 paraformaldehyde. 20 000 labelling events were routinely accumulated and analysed for fluorescence on FAS flow cytometer (FACscan, Becton Dickinson, USA) using Flow Max software. The results were statistically analysed using test ANOVA and Kruskal-Wallis. Results. Results are showed in the Table 1. Conclusion. We have demonstrated that there are no differences in distribution of CD158b and CD94:NKG2A on NK and CIsK cells in multiple myeloma and non-Hodgkin lymphoma patients.
CD49-NKG2A in NK and CJK in MM and nHL compared with normal donors. We have showed that the mean percentage of NK and CJK with CD49 expressing NKG2A is lower in MM patients compared with normal donors (p<0.05). It means that there is an increased expression of non-functional CD49 on NK and CJK of myeloma patients.

1119

UNEXPECTED ANTAGONISTIC EFFECT OF RITUXIMAB WITH PROCARBAZINE DISCLOSED DURING IN VITRO EVALUATION OF RITUXIMAB-MEDIATED SENSITIZATION OF B-CELL LINES TO COMMONLY USED ANTITUMOR DRUGS

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Backgrounds. Rituximab is a chimeric monoclonal antibody specific to the CD20-antigen expressed on mature B-lymphocytes. The antibody sensitizes lymphoma cells to differently acting cytotoxic drugs. Although some combinations of cytotoxic agents with rituximab have already been tested, there are many others for which no information is available. Aims. To analyse some commonly used and some new combinations of rituximab with differently acting cytotoxic agents in vitro using permanently growing B-cell lines. Methods. The stable cell lines derived from a follicular lymphoma (WSU-NHL), DHL-4, DOHH-2 and Burkitt lymphoma (RAMOS) were used for an in vitro viability assay. The cell lines were pretreated by 20 µg/ml of rituximab for 72 hours, followed by a subsequent incubation with the cytotoxic drugs (fludarabine, doxorubicin, vincristine, dexamethasone and procarbazine in four different concentrations) for 48 hours. A proliferation activity was estimated using a WST-1 assay. Obtained data were statistically evaluated using multi-way analysis of variance with interactions. The concentrations, presence or absence of pretreatment and plate variability were taken for the fixed effect. A cell cycle after the rituximab pretreatment was analysed by flow-cytometry with propidium iodide. Results. Rituximab significantly decreased an S-phase of the DHL-4 cells, while no prominent effect on cell cycle was observed for the other cell lines. We observed a significantly different sensitivity of follicular lymphoma and Burkitt’s lymphoma cells to vincristine and fludarabine (FL cells were highly sensitive to vincristine and rather poorly to fludarabine, while an opposite effect was seen for BL cells). The rituximab pretreatment sensitized all cell lines to vincristine, while none was sensitized to doxorubicin. Heterogenous results were obtained for the other combinations. A statistically significant influence of the rituximab pretreatment was proved for: dexamethasone at DOHH-2 and RAMOS, fludarabine at WSU-NHL and fludarabine and dexamethasone at DHL-4 cell lines. We obtained quite unexpected results for procarbazine in combination with rituximab. Although the drug strongly inhibited a metabolic activity in all tested cell lines, the effect was just opposite when the cells were pre-treated with rituximab. A highly statistically significant antagonistic effect was proved for all the cell lines. Summary. The data confirm that rituximab might sensitize lymphoma B-cells to most of differently acting anti-cancer agents. There are, however, some drugs manifesting a strong antagonistic effect with respect to rituximab. Therefore, based on our experimental data, the combination of rituximab with chemotherapeutic regiments containing procarbazine (e.g. R-COPP) does not seem to be clinically warranted.

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ASPIRIN RESISTANCE IN PATIENTS AFTER ISCHAEMIC STROKE AND ISOPROSTANE (8-EPITOUGASTRALGIN 2FA) PLASMA CONCENTRATION

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Backgrounds. Stroke may be partially related to aspirin resistance leading to continuous generation of intraplatelet thromboxane A2. Besides other underlying metabolic mechanisms, an oxidant stress along with nonenzymatic biosynthesis of isoeicosanoids supporting the platelet activation has been suggested. Aims. We analyzed the incidence of aspirin resistance in survivors of ischemic stroke and compared the usefulness of some platelet tests designed for its laboratory exploration. Methods. Forty four patients, at least a month after acute onset of ischemic stroke were included into the study. All of them have been receiving 75-150 mg aspirin daily at least for a month. The control group consisted of 12 adequately matched healthy volunteer. The platelet function was investigated by platelet aggregation induced by either ADP (5.5 and 5.0 µM), collagen (2 µg/ml or arachidonic acid (AA) (0.6 mM) and measurement of closure time on the collagen and epinephrine (Col/Epi) cartridge in PFA-100 analyzer. Thromboxane A2 metabolite - 11-dehydro Thromboxane B2 (11-dTXB2) and 11-epi F2α TM and 11-epi F2α TM (Col/Epi) closure time (t=0.36; p=0.019). Conclusions. 1. Laboratory tests reveal aspirin resistance in almost half of patients after ischaemic stroke. 2. The most significant correlation has been found between plasma concentration of reference indicator 11-dehydro Thromboxane B2 and PFA-100 closure time. 3. An important interrelationship observed between PFA-100 closure time and plasma concentration of 8-epi Prostaglandin F2α TM may support the hypothesis of nonenzymatic production of isoprostanoïds with platelet proaggregatory activity, playing a role in aspirin resistance.

1121

INDUCTION OF APOPTOSIS IN NB4 CELL LINE TREATED WITH ARSENIC TRIOXIDE AND THE EFFECT OF VIT.D3 ASSESSED BY THE COMET ASSAY

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Backgrounds. Successful treatment of acute promyelocytic leukemia (APL) relies on the ability to kill or arrest the growth of the leukemic blasts. This can be accomplished by inducing maturation, as is the case with the arsenic trioxide (in APL), and/or by inducing apoptosis. Various combinations of anti-leukemic agents (e.g. arsenic trioxide alone at the same concentration (p<0.05) in all groups. Conclusions. Results show that ATO induced apoptosis in NB4 cells at all doses used in this study. The effect was dose and time dependent and significantly different from controls (p<0.05). In contrast, Vit.D3 at concentrations of 100-600 nM showed no effect on induction of Apoptosis. Treatment of the NB4 cells with arsenic trioxide in combination with Vit.D3, a monocytic inducer, resulted in reduction of apoptosis as compared to arsenic trioxide alone at the same concentration (p<0.05) in all groups. Conclusions. Results show that ATO induced apoptosis in NB4 cells and the effect is dose and time dependent. On the other hand, the results suggest that Vit.D3 decreases the sensitivity of cells to arsenic trioxide. A significant decrease in apoptosis in the various treatment groups, clearly gives evidence that Vit.D3 has a protective role (in this combination). Also neutral comet assay can be considered as a suitable method for detection of chemically induced apoptosis.
A NOVEL T(14;17)(Q12;Q21) WITH REARRANGEMENT OF THE 17Q21 RARA LOCUS IN A CASE WITH JUVENILE MYELOMONOCYTIC LEUKEMIA

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Translocations involving the RARA locus on 17q21 have been identified in acute promyelocytic leukemia (APL). The majority of APL harbors the t(15;17)(q22;q21), resulting in a PML-RARA fusion transcript. Variant rearrangements involving RARA in APL are t(11;17) (q23;q21)[FLZ2-RARA], t(5;17)(q35;q21)[NPM-RARA], (t;11;17)(q13;21) (NUMA-RARA), a der(17)(STAT3b-RARA) and t(6;17) (p25;q21). Here we report a case of juvenile myelomonocytic leukemia (JMML) carrying a t(4;17)(q12;q21) with a rearrangement of the RARA locus at 17q21 demonstrated by FISH (probe LSI RARA DCBA, Abbott-Vysis). FISH analysis using BAC probes derived from the 4q12 region indicated that the breakpoint is located proximal of the CHIC and PDGFRα loci. The more proximally located HIP1L1 gene at 4q12 remains an attractive candidate gene. We will present ongoing FISH studies using probes spanning the 4q12 HIP1L1 locus and experiments to test whether the translocation results in HIP1L1-RARA fusion gene. This is the first report on a rearrangement of the RARA locus at 17q21 in JMML while now exclusively demonstrated in APL.

NUP98/HOMEOBOX HEMATOPOIETICALLY EXPRESSED (HHEX) FUSION GENE IN ACUTE MYELOID LEUKEMIA WITH T(10;11)(Q23;P15.5)

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Background. Chromosomal translocations involving the 5′ region of the nucleoporin gene, NUP98, on chromosome 11p15.5 emerged as recurrent leukemogenic events in myeloid and T lymphoid malignancies. Among the twenty partners identified so far there are both homeobox and non-homeobox, coiled-coil containing proteins. Aim. Identification of NUP98 recombinations in hematological malignancies with 11p15 partners. Methods. We performed cytogenetic and molecular studies in a 59-year-old man at second relapse of acute myeloid leukemia. Bone marrow karyotype was 46,XY,t(10;11)(q23;p15) in 15/15 metaphases. To study NUP98 involvement, metaphase FISH was done with the DNA clone RP5-1173K1 spanning exons 10 to 20 of NUP98. 3′ RACE-PCR experiments were performed using NUP_1083_F (5′-ggtaataccagcaccataggacag-3′) as a gene-specific primer. RT-PCR was performed with gene-specific primers NUP_1083_F (5′-ggtaataccagcaccataggacag-3′) and NUP_98_1861_R (5′-agc-gattctga-3′) to confirm the NUP98/HHEX chimeric transcript. The presence of NHEX/NUP98 chimeric transcript was investigated with HHEX_346_F (5′-ggacggtgaacgactacacg-3′) and NUP98_1861_R (5′-agc-gattctga-3′). The PCR products were subcloned in pGEM-T easy vector and sequenced. Results. RPS-1175SK gave three hybridization signals on normal 11, on der(11) and on der(10) indicating NUP98 rearrangement and the second NUP98 partner located more proximally ADD3 and t(10;11)(q23;p15)/NUP98-HHEX. Identification and characterization of NUP98 partner is an important step to unravel the leukemogenic mechanism(s) underlying NUP98 recombinations. Funding. Supported by MIUR, FIRB and Fondazione Cassa di Risparmio di Perugia.

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PROGNOSTIC SIGNIFICANCE OF CYTOGENETIC AND MOLECULAR CHANGES IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Chromosome aberrations and molecular rearrangements are closely associated with a particular morphologic or immunophenotypic subtype of childhood acute lymphoblastic leukemia (ALL). An integral part of diagnostic process in ALL patients is cytogenetic and molecular analysis. The aim of the study was to assess a prognostic value of genetic changes in childhood ALL. The total number of 70 children with newly diagnosed, untreated ALL, ranged from 1 month to 18 years of age, were included in the study. Conventional cytogenetics and RT-PCR analyses of fusion genes: BCR/ABL, MLL/AF4, E2A/PBX1 and TEL/AML1 were performed in all patients. Metaphase and interphase FISH technique with dual color translocation probes was used to verify the presence of fusion genes and to estimate the percentage of cells bearing them. To detect the rearrangements of MLL gene a split signal probe was used. Statistical analyses were performed with the help of Statistica program with Kaplan-Meier method and Cox multiple proportional hazard model. Chromosome preparations were obtained in 59 of 70 (84%) cases. 55 of 59 (90%) cases revealed chromosome aberrations. RT-PCR results were obtained in all 70 cases. Hyperdiploidy >50 chromosomes was present in 9 cases; in 6 cases only numerical (trisomies) and in 3 both numerical and structural aberrations were found. A hyperdiploidy 47-50 chromosomes was present in 6 patients, pseudodiploidy in 15 and hypodiploidy in 5. The fusion gene BCR/ABL was present in 2 out of 70 patients, PBX1/E2A in 2, and TEL/AML1 in 14. MLL/AF4 was not found but FISH with MLL split probe revealed the rearrangements of MLL (11q25) in 4 patients. The probability of event-free survival time (pEFS) and of total survival time (pST) was the highest in the groups of children with hyperdiploidy >50 chromosomes or with TEL/AML1 fusion, both without other changes, structural or numerical. Moreover, TEL/AML1 was a good prognostic factor only when present in a high percentage (>80%) of examined cells. The presence of pseudodiploidy or hypodiploidy correlated in general with moderate or poor outcome. The outcome for a group of patients with one of the following: t(1;19), t(9;22) or 11q23 rearrangement, was the worst and pEFS significantly lower than in the remaining patients (p<0.05). The most unfavorable independent risk factors were MLL rearrangements and BCR/ABL. The presence of MLL rearrangements caused 12-times increased, and BCR/ABL - 8-times increased risk of relapse or treatment failure. The WBC and early response to induction therapy were significant (p<0.05), independent hematological and clinical risk factors in ALL patients. The results of the study confirm the prognostic value of cytogenetic, FISH and molecular analyses in childhood ALL and underline the need of using them together at the diagnosis of ALL to establish the prognosis of the disease.

MOLECULAR ANALYSIS OF X LINKED CHRONIC GRANULOMATOUS DISEASE & MECLOD SYNDROME - A CASE REPORT

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Backgrounds. Chronic granulomatous disease (CGD) is a rare inherited disorder of the immune system, characterized by severe susceptibility to infections due to deficiencies in the phagocyte's ability to generate microbialic reactive oxygen species. Defects in any of the four components of the NADPH oxidase, [p47pox, p67pox,p22phox or gp91pox] a multicomponent enzyme complex, encoded by NCF1, NCF2, CYBA and CYBB genes respectively can give rise to CGD, the most common being the X-linked form of CGD due to mutations located on the X chromosome gene, Cytochrome b. We report a case of an Omani patient with X-Linked Chronic Granulomatous Disease. Methods. Clinical suspicion of CGD was confirmed with dihydrodorahminode reduction [DHR test] by FMA-stimulated neutrophils as an initial diag-
nostic test by Flowcytometry. Genomic DNA was isolated from peripheral blood leukocytes of the affected patient and two siblings and his father by QIAGEN DNA extraction Kit. Analysis for the presence of specific genomic coding sequences in several genes at the Xp21 locus namely DMD exon 59; PRRG1 exon 1; XK exons 1, 2, 3; CYBB exon 10; TCTE1L exon 5; SRPX exon 1; RGF1 exon 19 and OTX exon 1 were amplified by PCR reaction using specific primers. The presence of expected PCR products were reconfirmed by and documented by gel electrophoresis using an internal control gene. Additional studies were also performed to evaluate the Kell antigen system on the red blood cells. Results: DHR test showed no oxidative burst consistent with the diagnosis of CGD.

It was observed that the patient had a large deletion extending from PRRG1 to TCTE1L genes (Figure) with loss of both XK and CYBB genes. Flow cytometry showed weak expression of Kell antigens on the red blood cells of the patient. Summary/Conclusions: This study illustrates the rare event in our patient presenting with clinical manifestations of CGD and McLeod’s syndrome owing the underlying deletion of XK, CYBB, PRRG1 and TCTE1L genes.

**1126 IDENTIFICATION OF THE V617F JAK2 MUTATION IN MYELOPROLIFERATIVE DISORDERS**

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Backgrounds: Polycythemia Vera (PV), Essential Thrombocythemia (ET), Myelofibrosis (MF) and Chronic Myelogenous Leukemia (CML) were grouped into a spectrum of related disorders by Dameshek in 1951, dubbed Chronic Myeloproliferative Diseases (MPDs). However, a disease-causing genetic alteration (BCR/ABL rearrangement) has been identified only in CML. The other 3 disorders (PV, ET and MF) are classified as the BCR/ABL negative classic MPDs and are therefore distinguished from CML. The discovery of a single mutation in the Janus Kinase (JAK)-2 gene (substitution of a valine for a phenylalanine in the codon 617) in a high percentage of cases of PV, ET, MF suggests that it may be the underlying molecular mechanism for these disorders. This single mutation has been reported in 65-97% of patients with PV, 23-57% of ET cases, 35-57% of MF cases and in 20% of patients with unclassified MPDs.

The identification of JAK2 mutation represents a major advance in our understanding of the molecular pathogenesis of MPDs and provides a hallmark of genetic alteration in these disorders. Aims and Methods: We studied 57 Portuguese patients with MPD: 32 patients with MPD-NOS (not otherwise specified), 11 with PV, 11 with ET and 3 with idiopathic MF. In each case, DNA obtained from bone marrow or peripheral blood was amplified by PCR using specific primers for exon 12 of the JAK2 gene. The mutation V617F was detected by RFLP. Results: Analysis of 32 MPD-NOS revealed in 11 (46.9%) the V617F mutation. This mutation was also identified in 72.7% cases of PV and 27.3% of ET cases. V617F mutation was not identified in the 3 cases with idiopathic MF. Conclusions: The results of our study are in keeping with published reports, showing that V617F mutations are very frequent in MPDs, mainly in PV and in ET. These data suggest that the V617F mutations participate in the pathogenesis of chronic MPDs and will probably lead to a new classification of these diseases, contribute to a better stratification of patients according to prognosis, and hopefully allow the development of novel therapeutic approaches.

**1127 MOLECULAR DIAGNOSIS OF ß-TALASSAEMIA IN ROMANIA: THE FIRST APPLICATION TO PRENATAL DIAGNOSIS**

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Thalassaemia major is a classical example of a disease that can be prevented by prenatal diagnosis. In Romania there are currently 300 patients with thalassaemia major under the management of specialized institutions. So far, the prenatal diagnosis of thalassaemia was not available in Romania, for various reasons. For the prenatal diagnosis of ß-thalassaemia the first step is the characterization of the spectrum causing this disease in the Romanian population. In 2003 our institution, benefiting from the help of the Romanian Academy of Science initiated a Screening Programme for thalassaemia having as a main purpose to perform a screening for ß-thalassaemia mutations previously described in the Romanian population. METHOD: Haematological data were collected with automated cell counters (Coulter). Quantiﬁcation of haemoglobin was done by cation exchange HPLC and by agarose gel electrophoresis. Analysis of the mutation in the ß-globin gene has been performed by using the PCR based Methods. Amplification Refractory Mutation System (ARMS), restriction enzyme analysis and Denaturing Gradient Gel Electrophoresis (DGGE). Results: Until now we have identiﬁed 11 ß-thalassaemia alleles: IVS I-110 (37,88%), CD 39 (13,64%), IVS II-67 (16,64%), IVS II-745 (13,64%), IVS I-6 (13,64%), IVS 1-1 (7,58%), -87 (8,02%), CD 5 (3,03%), CD 6 (3,03%), CD51 (1,51%), +22 (1,51%), polyA (1,51%). Using this experience we were able to perform the ﬁrst prenatal diagnosis for a young couple at risk for thalassaemia major: maternal genotype IVS I’110 / Normal and paternal genotype IVS II’745 / Normal. Fetal samplings were collected as amniocentesis in the second trimester. Maternal contamination of the fetal DNA was ruled out by STR genotyping. Fetal genotype was IVS I-110 / IVS II’745 compatible with the presence of ß-thalassaemia major. These results were conﬁrmed by the DNA analysis performed in National Thalassaemia Center from Athens, Greece. CONCLUSION: The results of this study point to a successful future prenatal diagnosis of ß-thalassaemia in Romania, using a rapid and accurate molecular method. Together with the implementation of proper preventive health measures and the education of the parents regarding their carrier status, we are hoping that this method will be used as the common application approach to decrease the incidence of thalassaemia major.

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**1128 DEVELOPMENT OF A QUANTITATIVE METHOD FOR ASSESSMENT OF BCR-ABL TRANSCRIPTS BY TAQMAN TECHNOLOGY FOR THE LIGHTCYCLER INSTRUMENT**


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The quantification of BCR-ABL transcripts is mandatory for the follow-up of patients according to prognosis, and hopefully allow the development of novel therapeutic approaches.
low-up of patients with chronic myeloid leukemia who are treated with imatinib. Hematopoietic stem cell transplant is currently accomplished by means of various fluorescence-based detection techniques which allow for the monitoring of the amplification process in the so-called real-time PCR. The recent availability of instruments for real-time quantitative PCR (RT-PCR) has prompted the development of quantitative methods for the most common fusion transcripts detectable in hematologic malignancies. However, because the ABI PRISM apparatus (Applied Biosystems) was the first available device for real-time PCR, most of the assays were developed with the use of the TaqMan probe chemistry. Upon introduction of other real-time PCR instruments, such as the LightCycler (LC; Roche), different methodologies were described. The aim of this study was to optimize the protocol established for ABI PRISM by J. Gabert et al. under the Europe Against Cancer Program (EAC Protocol, April 2002) for use with the LC. In addition, the aim was to establish a standard approach of fluorescence data acquisition. The reverse transcription protocol was applied with the use of Superscript reverse transcriptase, starting from a total RNA amount extracted from at least 5x10^6 cells. The PCR reaction mix for the BCR-ABL was prepared in a final volume of 20 µL containing 300ng of each primer, 200 nM of the ABL, TaqManTM probe, 2 µL of FastStart DNA Master Hybridization Probes (Roche), 3 mM MgCl2, 1 unit Uracil-DNA glycosylase and 5 µL of DNA from patient samples or plasmid DNA dilutions for the creation of the standard curve. The PCR reaction mix for the ABL contained 300ng of each primer and 200nM of the ABL. TaqManTM probe. The LC PCR program consisted of an initial denaturation at 95°C for 12 min, followed by 45 cycles of 95°C for 10 sec, and 60°C for 60 sec with fluorescence reading at F1 channel. Data analysis was performed in the F1/F2 mode and quantification was obtained by selection of the fit points option. We adjusted the noise band at 0.02 to eliminate background signals. For the analysis step, the crossing line was set at 0.1. In 47 consecutive experiments, the median ± SD of the slope of the calibration curve for BCR-ABL and ABL was 3.55±0.16 and -3.57±0.29 respectively. The median±SD of the PCR efficiency for BCR-ABL and ABL was 1.91±0.06 and 1.92±0.11 respectively. The median ± SD of the intercept for BCR-ABL and ABL was 40.09±1.19 and 41.48±1.6 respectively. Consequently, the values of the above parameters, that determine PCR efficiency, were within the acceptable limits as defined by the EAC Protocol. The variation in Cq values was less than 1.5 (when Cp value <30) indicating the reproducibility of the RQ-PCR analysis. In conclusion, the TaqMan technology-based protocol can be successfully applied to the LC device with the proper fluorescence data acquisition method, which provides accurate quantification of BCR-ABL transcripts in accordance with the guidelines of the EAC Protocol.

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TRANSCRIPTIONAL PROFILING OF EPSTEIN-BARR VIRUS (EBV) GENES AND HOST CELL GENES IN NASAL NK/T-CELL LYMPHOMA AND CHRONIC ACTIVE EBV INFECTION

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Backgrounds. Nasal NK/T-cell lymphomas an aggressive subtype of non-Hodgkin lymphoma (NHL) that is closely associated with Epstein-Barr virus (EBV). The clonal expansion of EBV-infected NK or T cells is also seen in patients with chronic active EBV (CAEBV) infection, suggesting that two diseases might share a partially similar mechanism by which EBV affects host cellular gene expression. Aim. To understand the pathogenesis of EBV-associated NK/T-cell lymphoproliferative disorders (LPD) and design new therapies. Methods. We employed a novel EBV DNA microarray (HHV-4 Viruchip) to compare patterns of EBV-expressed genes in cell lines established from EBV-associated NK/T-cell LPD. We also analyzed the gene expression patterns of host cellular genes using an Affymetrix U133plus2.0 chipset. Results. We found that expression of BZLF1, which encodes the immediate early gene product Zta, was expressed in SNK/T cells. We identified a subset of pathogenetic features of NK/T cells that could identify novel therapeutic targets in EBV-associated NK/T-cell LPD.

1330

GENE EXPRESSION PROFILING AND DETERMINATION OF GENETIC HETEROGENEITIES AS PROGNOSTIC FACTORS IN PATIENTS WITH MULTIPLE MYELOMA TREATED WITH CONVENTIONAL VERSUS NOVEL AGENTS IN CORRELATION WITH OUTCOME

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The standard treatment of newly diagnosed multiple myeloma (MM) patients is based on induction treatment followed by high-dose melphalan. CR/nCR percentages range from 20-50% with event free survival (EFS) ranging from 18 months to 20 months. The 5-year survival rates are 25% to 50%, however all patients eventually relapse and succumb to the disease. In patients with unfavourable prognostic factors such as a high serum β2-microglobulin and/or a deletion of chromosome 13, or in elderly patients the prognosis remains poor. Recently, several promising new agents were developed, which interfere with critical cell-survival pathways in myeloma. Amongst these agents, Bortezomib, a proteasome inhibitor, and Thalidomide, an anti-angiogenic and immunomodulatory drug, have a remarkable effect in patients with relapsed or refractory MM with 30-40% response rates. In combination with Dexamethasone and/or other conventional agents overall response rates of 50-70% can be achieved. In newly diagnosed patients the response rates vary from 70 - 85%. Moreover, Bortezomib was found to overcome poor prognostic factors like a high β2-microglobulin and/or deletion of chromosome 13. However, 15-30% of newly diagnosed patients do not respond to Bortezomib or Thalidomide. Secondly, 30% of the patients treated with these novel agents have to stop prematurely because of intolerable side effects, such as polyneuropathy, thrombocytopenia, thrombosis and gastro-intestinal symptoms. To gain new insights into the mechanisms of drug response and toxicity associated with these agents, we have embarked on a prospective study to analyze gene expression profiles of myeloma specific genes in plasma cells purified from bone marrow from myeloma patients at diagnosis who have been treated with these novel agents in order to learn which genes govern the response, PFS and OS upon treatment with Bortezomib and Thalidomide. Gene expression profiling of CD138 magnetic cell selected (MACS) myeloma plasma cells will be performed using Affymetrix GeneChip Human Genome U133 plus 2.0 arrays. In addition, we will perform a Single Nucleotide Polymorphism analysis of germline DNA on myeloma patients is based on induction treatment followed by high-dose therapy with stem cell rescue and maintenance therapy with Bortezomib and Thalidomide. Gene expression profiling of CD138 magnetic cell selected (MACS) myeloma plasma cells will be performed using Affymetrix GeneChip Human Genome U133 plus 2.0 arrays. In addition, we will perform a Single Nucleotide Polymorphism analysis of germline DNA on myeloma patients is based on induction treatment followed by high-dose therapy with stem cell rescue and maintenance therapy with Bortezomib and Thalidomide. Gene expression profiling of CD138 magnetic cell selected (MACS) myeloma plasma cells will be performed using Affymetrix GeneChip Human Genome U133 plus 2.0 arrays.
Treating HL 60 cells with docetaxel and 20 patients were found to be as effective as taxol. Western blotting with specific antibodies against protein phosphatases was used to determine the changes in the expression of protein phosphatases after incubation of cells with taxans. Protein phosphatase activities were assessed by using specific ELISA kits. Results. Treating HL 60 cells with docetaxel and paclitaxel resulted in dose and time dependent cytotoxicity with 24 hours intervals. Combination studies of these drugs with phosphatase inhibitors showed significant increase in the taxan-induced cytotoxicity of HL 60 cells. Acridine orange/ethidium bromide and Hoechst 33342-PI methods confirmed the taxan-induced apoptosis of leukemic cells. Protein phosphatase 1 and 2A activity was found to be increased after treating cells with docetaxel and paclitaxel at maximum level of 72-hour. Western blotting results showed the increase in the expression of protein phosphatase 1A catalytic subunit at 72-hour of incubation. Conclusion: Serine/threonine protein phosphatase system has significant role in taxan-induced cytotoxicity against leukemic cells. Potential use specific protein phosphatase inhibitors in combination with taxans will open new windows in the treatment of myeloid leukemias.

**1132**
A NEW FORMULA FOR DIFFERENTIATION OF IRON DEFICIENCY ANEMIA (IDA) AND THALASSEMIA TRAIT (TT)
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Introduction: The most commonly encountered disorders with mild microcytic anemia are iron deficiency anemia and thalassemia trait. Sensitivity and specificity of many discriminate indices have been reported using red blood cell indices. Youden’s index provides an appropriate measure of validity of a particular technique or question by taking into account both sensitivity and specificity. We compare the Youden’s index for these indices as well. Methods. We studied 284 individuals with microcytic anemia aged between 6 month and 75 years. There were 188 females and 96 males involved in our study with mean age equal to 24.23(SD, 15.44). Ferritin, HbA2, and Complete Blood Cell (CBC), in which RBC, hemoglobin (Hb), Hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin(MCH), and Mean Corpuscular hemoglobin concentration(MCHC), were measured for all the participants. We diagnosed individuals with HbA2>3.4% as patients with β-thalassemia (βTT) and those who have a serum ferritin <12ng/mL or respond to administered Iron and anemic situation in their blood supply as patient suffering from Iron deficiency anemia (IDA). England Index, Mentzer Index, Srivastava Index, Kawakami Index, have been calculated for all formulas as well as OUR INDEX (Ehsani Index = MCV x (RBC) 109/RBC) Blood Counts were obtained by H1 Technicon Cell Counter System while ferritin was measured and HbA2 value determined by electrophoresis. Sensitivity, specificity, Positive IDA Predictive Value (PPV), Negative IDA (BTT) Predictive Value (NPV), and Youden Index (YI) was calculated. Results: Considering the above criteria we diagnosed 130 patients with BTT and 154 patients with IDA. Sensitivity and specificity for England Index was 99.2 and 69.5, Mentzer Index 94.6 and 95.5, Srivastava Index 88.5 and 85.7, Kawakami Index 86.2 and 98.1, and Ehsani’s Index 90.0 and 95.5. Conclusions: The most frequently encountered diseases with microcytic anemia are TT and IDA. Screening for TT is of great importance in order to address the patient to a genetic counselor. Iron should not be administered to patients with TT as an attempt to normalize MCV so differentiating TT from IDA has a great importance. Decreased levels of SI, TS and ferritin with increased levels of SBC are the main diagnostic criteria for IDA. The diagnosis of BTT is established by the presence of characteristic red blood cell microcytosis and elevated levels of HbA2. However in some mutations of BTT and in heterozygous α thalassemia, HbA2 is not elevated. The use of Ehsani Index is so easy and anybody can subtract the ten fold RBC from MCV in mind with no need for calculator.

**1133**
α THALASSEMA IN BAHRAIN
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α-thalassemia is one of the commonest genetic disorders in the Arabian Gulf region and its reported incidence varies from 15% to 50% in the different countries in this region. Despite this widespread occurrence there are few comprehensive studies that describe genotype-phenotype correlations in this disorder as observed in this geographic location. To correlate the genotypes of α-thalassemia in Bahraini subjects with the respective phenotypic characteristics. Forty α-thalassemia cases were selected from patients referred by participating hematologists for the investigation of anemia or unexplained microcytosis. The following tests were done for each patient: (i) measurement of hemoglobin and red cell indices (ii) staining for HbH inclusions (iii) analysis of hemoglobin and quantitation of hemoglobin fractions by HPLC and (iv) molecular genotyping using a PCR-based strategy to identify the four common α-thalassemia haplotypes prevalent in the region (α2-, α2α, α2αHptx and α2Saud). The assessment of clinical severity was based on the degree of anemia, the requirement for and number of episodes of transfusions during the stay of treatment and age at first transfusion. The αSaud/Hptx haplotype was the most common with a frequency of 41.9% among all haplotypes. This was followed successively by α2α/α2α (37.8%), α2αHptx/α2α (10.8%) and α2α/α2αHptx (9.5%). The homozygous αTSaudi genotype was characterized by presence of high numbers of cells containing intraerythrocytic inclusions of HbH with typical morphological, markedly altered erythrocyte indices especially the RDW, high levels of deoxyhemoglobin (Hb Bart’s and/or HbF) and greater clinical severity. The other genotypes showed overlapping phenotypic features but none were severely affected. The homozygous αTSaudi abnormality is the only genotype in Bahrain with a distinctive phenotype that is identifiable by routine laboratory tests and accounts for almost all the severely affected cases. Premarital screening programs in the region should take these considerations into account when screening strategies are formulated.

**1134**
EFFICACY OF HYDROXYUREA (HU) IN REDUCTION OF PACK RED CELL TRANSFUSION REQUIREMENT AMONG CHILDREN HAVING β-THALASSEMA MAJOR: KARACHI HU TRIAL (KHT)
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Bismillah Taqee Institute of Health Sci., KARACHI, Pakistan

Backgrounds. PRC transfusion and iron chelation remains the mainstay of treatment of β-thalassaemia major patients. HbF augmentation is the exciting new approach to treat haemoglobinopathy. Aims. This study evaluates the efficacy and safety of HU to reduce the volume of PRC transfusion in β-thalassaemia. Methods. 23 patients with β-thalassaemia major received HU mean dose, 16mg/kg/day. The results were analyzed at the end of 24 months. Transfusion requirement 6 months before starting HU was considered as control. Results. 20 patients were evaluable after 24 months. Mean volume of PRC transfused was reduced in all. Mean PRC requirements for six months before starting HU was 2126.45 mL where as after 24 months on HU was 1489.59 ml (mean difference: 637.3 mL; 95% CI: 402.8 - 817.8; p<0.001). Interval between transfusions was increased by 68.7%. Mean increase was 12.1 days (CI: 18.0 - .45; p value: <0.001). Statistically insignificant increase was noted in ferritin levels with mean difference of 657.1 ng/L (95% CI: -1475.3, -18.0, -6.3; p value: 0.001). Conclusion. Hydroxyurea was found to be a safe medicine in β-thalassaemia. It showed a reduction in transfusion requirement and increased interval between PRC transfusions.

**1135**
ENDOGENOUS ERYTHROPOIETIN PRODUCTION AND ERYTHROPOIETIC ACTIVITY IN ANEMIC CANCER PATIENTS WITH HEMATOLOGICAL MALIGNANCIES
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1Clinic for hematology, SKOPE, Macedonia; 2Clinic for Hematology, SKOPE, Macedonia

Backgrounds. Cancer anemia is multifactorial: blunted erythropoietin...
(Epo) response has also been encountered. As it is particularly well documented in some types of anaemia of chronic disease (ACD), we investigated the Epo deficiency in anemic cancer patients with hematologic malignancies. Patients and Methods. 42 patients (pts) with multiple myeloma (MM), 27 pts with malignant lymphoma (ML) and 19 pts with chronic lymphocytic leukemia (CLL) were included in the study. 25 pts with iron deficiency anaemia represented the control group. Serum Epo and serum transferrin receptors (sTfR) were measured with commercially available assays. O/PEpo and O/PsTfR ratios (O-observed value, P-predicted value) were derived for each patient in order to assess if Epo response and erythropoietic activity are appropriate for a given degree of anaemia. Predicted values were calculated from the regression equation of serum Epo, respectively sTfR versus hemoglobin (Hb) determined in the control group. A correlation between O/PEpo and O/PsTfR was searched in order to assess the impact of Epo deficiency on erythropoietic activity and therefore the anaemia. Results. All pts, except for the MM pts with renal failure showed Epo response to anemia: a significant inverse correlation between serum Epo and Hb was found. Epo production and erythropoietic activity as determined from the control group were inappropriate if the values for O/PEpo and O/PsTfR were ≤0.8 and 0.9, respectively. 48% pts with MM and no renal failure, 33% pts with ML and 11%pts with CLL had inappropriate Epo response to anaemia. Erythropoietic activity was inappropriate in 76% pts with MM, 48% pts with ML and 87% with CLL. The inappropriate erythropoietic activity and therefore the anaemia were significantly influenced from the inappropriate erythropoietin production: this could be shown by the positive correlation between O/PEpo and O/PsTfR in all three patient groups. Conclusion. Significant number anemic pts with hematologic malignancies have blunted Epo response to anaemia. The adequacy of serum Epo levels could be convincingly assessed by O/PEpo and O/PsTfR ratio and should be used to predict the therapy response to HU-Epo in anemic cancer pts.

**The Safety of Avoiding Pre-Operative Transfusion in Patients with Sickle Cell Anemia**

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Backgrounds. Sickle cell anemia (SCA) is a common hereditary blood disease seen in Saudi Arabia, the affected patients associated with severe clinical manifestations. It is generally recommended for patients with sickle cell anemia to receive red blood cell (RBC) transfusions before undergoing general anesthesia and surgery. Patients with sickle cell anemia have increased chance of undergoing surgical procedures with higher morbidity. The practice of preoperative blood transfusion for such patients is still controversial. Lately, a great deal of controversial data accumulated in regards to transfusion management of such patients who require surgery. Aim. The aim of this prospective study was to assess the role of pre-operative transfusion practice in patients with SCA, whether or not omissions of such preparation lead to complications.

<table>
<thead>
<tr>
<th>Complications</th>
<th>Group I (N=181)</th>
<th>Group II (N=188)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Painful Crises</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Neurological Complication</td>
<td>0</td>
<td>4*</td>
<td></td>
</tr>
<tr>
<td>Minor Respiratory Complication</td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Respiratory Distress (Atelectasis)</td>
<td>0</td>
<td>3*</td>
<td></td>
</tr>
<tr>
<td>Circulatory Overload or Heart failure</td>
<td>0</td>
<td>5*</td>
<td></td>
</tr>
<tr>
<td>Infection</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Total (Percentage)</td>
<td>13 (7.0%)</td>
<td>27 (14.0%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Dealy of Surgery</td>
<td>1</td>
<td>45**</td>
<td>&gt; 0.001</td>
</tr>
</tbody>
</table>

* Hemoglobin ≥ 10.5 g/dL; ** To fulfill the criteria of Hb S concentration ≤ 40% pre-operative.

Methods. A randomized study of 369 patients, median age 16-years-old (range: 1-85 years old), during the period between June 1996 and June 2001, underwent different surgical procedures at King Abdulaziz University Hospital and King Fahd Armed Forces Hospital. Surgical procedures included adenoidectomy, tonsillectomy, total lrp arthroplasty, cholecystectomy, splenectomy, and Obstetric and Gynecological surgery. Patients were randomized into two groups: Group I (n=181), received no preoperative transfusion but were transfused compensated for blood loss during surgery. Group II (n=188) received simple or partial exchange transfusion preoperatively. All patients were clinically and hematological stable in the immediate pre-operative period; also, were carefully hydrated and good oxygenation was maintained. Results. Results showed none of the patients developed major intra- or post-operative complications in both groups. 14.4% of the preoperative transfusion group developed post-operative complications in comparison to 7.2% in non-transfused group with a significant p value (0.002). Conclusion. Avoidance of preoperative transfusion is a safe practice in properly selected steady state sicklers. On the contrary, it is believed that the risks associated with transfusion were avoided.

**Pharmacokinetics of Erythropoietin Produced by a Human Cell Line (EPOETIN Δ) Subcutaneous vs. Intravenous Dosing in Patients with Chronic Kidney Disease**

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Backgrounds. Epoetin Δ (Dynepe-TM, Shire) is an erythropoietin produced by gene-activation technology in a human cell line. As a result, it contains very few of the highly immunogenic NεGc residues that are more commonly found in other recombinant erythropoietins. In renal anaemia, the preferred route of administration for erythropoietin (either subcutaneous [sc] or intravenous [iv]) often differs depending on the status of the patient, the erythropoietin used and local practice. Aims. To assess the pharmacokinetics of iv or sc epoetin delta in patients with anaemia and end-stage renal disease requiring dialysis. Methods. Patients with end-stage renal disease requiring dialysis who had been receiving epoetin α for at least 90 days entered a 1-week washout phase during which they did not receive recombinant erythropoietin. Patients were then randomized to one of four groups receiving single doses of epoetin delta 150 IU/kg (iv or sc) or 300 IU/kg (iv or sc). Blood samples were drawn before and for 72 h after administration. Results. In total, 28 patients entered the washout phase and 22 of these (12 men, 10 women) went on to receive epoetin Δ and complete the study. Pharmacokinetic parameters are shown in Table 1.

**Bioavailability of sc epoetin Δ was 26.86% of that of iv epoetin Δ. The half-life of sc epoetin delta was approximately 30 h compared with 10-13 h for iv epoetin Δ. Treatment-emergent adverse events occurred in 45% of patients, but none of these were considered by investigators to have any relation to epoetin Δ. Conclusions. As expected, the pharmacokinetics of iv and sc epoetin delta differ depending on route of administration. The half-life of epoetin Δ in patients with renal anaemia may be slightly higher than that reported for epoetin α (a half-life as low as 4 h has been reported), suggesting that longer dosing intervals may be possible with this agent.**
1138
TWO NOVEL G6PD VARIANTS, G6PD PEDOPIS-CKARO AND G6PD PIOTRKOW IN POLAND
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Backgrounds. Glucose-6-phosphate dehydrogenase (G6PD) is the key enzyme of the pentose phosphate pathway whose main physiological function in red blood cells is to produce the NADPH, essential for the protection of the cells against oxidative stress. The majority of people with G6PD deficiency are asymptomatic but they may develop acute hemolytic anaemia in association with infections or following the ingestion of certain drugs or fava beans. In some sporadic cases G6PD deficiency is the cause of chronic non-spherocytic haemolytic anaemia, CNSHA. Aims. The aim of our study was to elucidate the molecular basis of G6PD deficiency. Methods. Genomic DNA was extracted from peripheral blood using standard methods. G6PD gene exons 2 to 13 were amplified by polymerase chain reaction (PCR). DNA fragments generated by PCR amplification were directly sequenced. The appropriate restriction enzyme analysis was used to verify the presence of found mutations. Molecular modeling of the tertiary structure of the G6PD molecule was used to check the influence of these mutations on the enzyme structure. Results. Direct sequencing of G6PD gene of two compensated G6PD-deficient patients showed two novel mutations: 573C→G mutation which is located in exon 6 and results in β-subunit mutation named G6PD Pedopis-Ckaro, and 2470G→A, resulting in H296Q mutation; the second patient was named G6PD Piotrkow respectively, after the patients’ place of origin. The results of molecular modeling correlate with clinical picture of studied patients.

1139
BASELINE IRON STUDIES DEMONSTRATE SEVERE IRON OVERLOAD IN PATIENTS ENROLLED INTO THE DEFERASIROX (EXJADE, ICL670) CLINICAL TRIAL PROGRAMME
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Backgrounds. Iron overload is a potentially life-threatening consequence of regular administration of the reference standard chelator deferoxamine (DFO, Desferal®), and therefore do not gain the full benefits of treatment. Deferasirox (Exjade, ICL670) is a novel, once-daily, easily applicable, oral iron chelator that is currently approved for use in eight countries, including the USA and Switzerland, in patients aged ≥12 years with chronic transfusional iron overload. To date, more than 1,000 patients have been enrolled into the deferasirox clinical trial programme. Aims. The primary aim of this cross-study analysis was to evaluate the severity of iron burden in patients prior to enrolment into the deferasirox clinical trial programme. Methods. Liver biopsy was performed at baseline and after 1 year in patients participating in two deferasirox clinical studies, 107 (n=495) and 108 (n=120). In Study 107, 248 patients with β-thalassemia were randomly assigned to deferasirox (5, 10, 20 or 30 mg/kg/day) and 247 to DFO (<25, 25–<35, 35–<50 and ≥50 mg/kg) according to baseline liver iron concentration (LIC). In Study 108, 67 patients with β-thalassemia and 53 with other anaemias (eg myelodysplastic syndromes, Diamond-Blackfan anaemia, rare anaemias) were enrolled and received deferasirox or DFO. Results. In general, baseline characteristics were comparable between deferasirox and DFO cohorts. Paediatric patients (aged <16 years) comprised 38% of the study population. Mean baseline LIC was high in the overall population (Table 1), with approximately 80% of patients having a baseline LIC ≥7 mg Fe/g dw and the majority (68.6%) ≥20 mg Fe/g dw. Published data have linked LIC levels above ≥7 mg Fe/g dw with an increased risk of developing iron overload-related complications, primarily heart-related. Baseline serum ferritin levels were also very high and above clinically acceptable values. For most patients, transfusional iron intake was 0.30–0.5 mg/kg/day, corresponding to a mean daily amount of blood given of 0.85 ± 0.11 mL RBC/kg. In addition to this global analysis, local analyses by country have been performed. Conclusions. Baseline iron burden, as reflected by LIC and serum ferritin levels, was very high and above published clinically acceptable thresholds. This analysis demonstrates that despite the availability of chelation therapy, many patients were severely iron overloaded and therefore at high risk for developing complications. There were no differences between adult and paediatric patients. This suggests that patients were not receiving adequate chelation therapy to achieve iron balance. The development of a highly efficacious, well-tolerated and convenient iron chelator will improve compliance and allow physicians to use an effective chelation programme for their patients. This was a primary goal of the rigorous development programme for the once-daily, oral chelator deferasirox, which culminated in its registration with a broad indication by a number of health authorities.

Table 1. Patient demographics and baseline characteristics.

<table>
<thead>
<tr>
<th>All patients (n=615)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, years</td>
</tr>
<tr>
<td>Patients aged 2 to &lt;16 years, n(%)</td>
</tr>
<tr>
<td>Patients aged ≥16 years, n(%)</td>
</tr>
<tr>
<td>Male: Female</td>
</tr>
<tr>
<td>Mean baseline LIC≤SD, mg Fe/g dw</td>
</tr>
<tr>
<td>All patients</td>
</tr>
<tr>
<td>2 to &lt;16 years</td>
</tr>
<tr>
<td>≥16 years</td>
</tr>
<tr>
<td>Baseline LIC category, n(%)</td>
</tr>
<tr>
<td>&lt;7 mg Fe/g dw</td>
</tr>
<tr>
<td>7 to &lt;10 mg Fe/g dw</td>
</tr>
<tr>
<td>≥10 mg Fe/g dw</td>
</tr>
<tr>
<td>Mean baseline serum ferritin ≤5SD, mg/mL</td>
</tr>
<tr>
<td>All patients</td>
</tr>
<tr>
<td>2 to &lt;16 years</td>
</tr>
<tr>
<td>≥16 years</td>
</tr>
<tr>
<td>Mean transfusional iron intake ≥5SD, mg/kg/day</td>
</tr>
<tr>
<td>Iron intake category n (%)</td>
</tr>
<tr>
<td>0 to &lt;0.3 mg/kg/day</td>
</tr>
<tr>
<td>0.3–0.5 mg/kg/day</td>
</tr>
<tr>
<td>&gt;0.5 mg/kg/day</td>
</tr>
<tr>
<td>Mean blood given ≥5SD, mL RBC/kg/day</td>
</tr>
</tbody>
</table>

1140
DETECTION OF RARE RHCE ALLELES IN COMORIAN INDIVIDUALS LIVING IN MARSEILLES, FRANCE
M. Touinssi, P. Bailly, J. Chiaroni
EFS-Alpes Mediterranée, MARSEILLES, France

Background. More than 70 000 comorians are living in Marseilles at present (10% of the total Comorian population has immigrated to France since 1970). Due to their genetic background and lack of data on this population, some difficulties of transfusion are encountered. As described previously (Nacaz et al. Blood, 2002), some rare RHCE phenotypes are found exclusively in black populations: i) RH-18 (712A→G) with the three alleles (ceE1, ceA1, ceB1), ii) RH-54 phenotype is produced by the (C)ces haplotype, iii) Partial Rhe: produced by the new ces(340) allele carrying an extra-mutation in exon 3 (340C→T) and by the cE0M0 allele. Aims. The aim of this work was to detect rare RHCE alleles, in Comorians of Marseilles, and to supply data on this population. A cohort of 260 unrelated immigrants of both sexes living in Marseilles France, participated to this study and were considered as representative of Comorian population, particularly, Grande Comore. This study was approved by the competent authorities in France (CCPRB n°00/48). Methods. Genomic DNA was isolated from peripheral blood leukocytes using a Qiamp Blood DNA Mini kit (Qiagen®, Hilden, Germany). Allele-specific

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ic primer PCRs were used to detect specific mutations corresponding to RHR1, RHR2, or RHR3. If no mutations were found, RHR-PCR was then conducted to determine their homozygous or heterozygous status. Results: Six individuals were found positive for the 712A→G mutation. Sequencing of RHCE exons 4, 5 and 6 showed that five individuals were heterozygous for ceAR and one was heterozygous for ceKE. 736C→G mutation, associated with antigens RH10 and RH20, was observed with high frequency (54% were positive and 58% were homozygous or heterozygous). Two of those individuals carried RHR2 mutation, while one carried 306A→G mutation. Conclusions: These findings broaden the genetic basis of RHR types and allow better understanding of the phenotypic variability associated with these antigens.

**1142**

MEASUREMENT OF ERYTHROCYTE BAND 3 EXPRESSION IN HEREDITARY SPHEROCYTOSIS

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**Background.** Hereditary spherocytosis (HS) is the most common inherited anemia among the spherocytic anemias, whereas autoimmune hemolytic anemia is the most common acquired. The prevalence of HS is not known, partly because of the heterogenous clinical manifestations. The most specific diagnostic techniques, i.e. erythrocyte protein analysis and molecular genetics are only provided by a few reference laboratories. In the routine setting diagnosis is based on typical family history, splenomegaly and jaundice and the finding of spherocytes and reticulosis in the blood and increased osmotic fragility of the erythrocytes. The red cell anion exchanger band 3 is present in great numbers in the erythrocyte cell membrane. In HS the expression of band 3 is diminished irrespective of the primary protein defect. The band 3 expression can readily be measured by flow cytometry after labelling with eosin-5maleimide (EMA) (King et al. 1994). Aim: Determine the diagnostic characteristics of the flow cytometric band 3 expression test.

**Methods.** We have measured band 3 expression in 50 patients with HS, 30 patients with other hemolytic anemias and in 200 healthy volunteers. We have also studied band 3 expression in patients with autoimmune hemolytic anemia, G-6-P-deficiency, PK-deficiency, sickle cell anemia and other rare forms of anemia. We have also studied the influence of recent transfusions and ongoing profuse hemolysis. Results. In our laboratory the cut off value for band 3 expression for diagnosing HS is 92.5% of that in non-HS persons. We confirm the findings of others that the band 3 expression is normal in all other forms of anemia than HS. Ongoing hemolysis and recent transfusions can diminish the sensitivity of the method. Conclusion. The flow cytometric measurement of band 3 expression has a high sensitivity and specificity for diagnosing hereditary spherocytosis and should be one of the primary investigations in cases of suspected hereditary spherocytosis.

**1143**

GLUCOSE TOLERANCE IN PATIENTS WITH β-THALASSAEMIA MAJOR

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**Background.** In chronically transfused thalassemic patients, whether insulin resistance is the primary abnormality leading to glucose metabolism disturbances, or early introduced pancreatic damage and reduced insulin secretory capacity is the main cause of glucose intolerance, remains uncertain. Aim: To examine the incidence of glucose disturbances and endocrine co-morbidity in transfused patients with β-thalassaemia major. Methods: We assessed glucose responses during an oral glucose tolerance test in 243 regularly transfused thalassemic patients (107 males, 136 females, 25.26 ±6.2 years, age ± standard deviation, range 13-48). Patients’ records were thoroughly reviewed to determine the overall transfusional iron overload and start of chelation therapy (age and time of first blood transfusion and start of iron chelation). Results. Patients were stratified according to the criteria of the American Diabetes Association in three subgroups: Normal glucose tolerance (NGT, n=197, 81%) impaired glucose tolerance (IGT, n=25, 10,3%) and diabetes (n=21, 8,7%). There were no differences between the groups regarding the parameters of transfusion/Chelation therapy. However, the development of glucose intolerance was significantly increasing with age (p<0.001). Except hypothyroidism, all the other endocrine complications were significantly more frequent in patients with diabetes and IGT (Table).
Conclusions. The degree of iron overload, at least as this can be reflected by ferritin levels, is not associated with the development of glucose intolerance. Long-term iron balance rather than the present iron status seems to be related to the development of glucose metabolic disorders. Physicians caring for patients with thalassemia major should be particularly alert to glucose intolerance since co-existence of other endocrine complications is common in these patients.

1144 EVOLUTION OF THROMBOCYTOSIS RELATED TO IRON DEFICIENCY ANEMIA
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1Hopital Farhat Hached, SOUSSE, Tunisia, 2 Hopital Farhat Hached, SOUSSE, Tunisia

Backgrounds. The incidence and the outcome of thrombocytosis related to iron deficiency anemia is not described very well. The aim of this study was to perform an analysis of the evolution of thrombocytosis related to iron deficiency. We performed a retrospective analysis of 1570 consecutive patients with iron deficiency anemia collected between 1995 and 2005. Four hundred 40 patients (29%) had a thrombocytosis more than 500x109/L. They were between 18 months and 85 years old. The mean of hemoglobin was 58 g/L and the mean of platelets was 745 ×109/L. There was no correlation between the importance of anemia, iron deficiency and the importance of thrombocytosis. The evolution was favourable in all cases with correction of thrombocytosis with a median of 16 days. There was not any thrombo-embolic complication. We conclude that thrombocytosis is frequently associated to iron deficiency anemia and the outcome is always favourable with a rapid correction and without risk of thrombo-embolism.

1145 EFFICACY OF IRON-CHELATION THERAPY IN BULGARIAN CHILDREN WITH β-TALASSEMA MAJOR
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1Thalassemia Centre, Children’s Universit, SOFIA, Bulgaria, 2Aleksandrovska Hospita, SOFIA, Bulgaria

Backgrounds. Iron-chelation therapy is an essential part of clinical management of patients with β-thalassemia major (TM). It decreases iron-induced organ damage and provides longer survival of thalassemics. Deferoxamine (DFO) and deferoxiprone (L1) are two iron chelators available now in most of the countries. Aims. To evaluate the efficacy and safety of iron-chelation therapy in Bulgarian pediatric patients with TM. Methods. 85 children with TM (age 8-16 years) were included. They were treated with DFO alone (DFO administered subcutaneously 40 mg/kg 5 days/week in 30 children) or DFO in combination with L1 (DFO subcutaneously 40 mg/kg 5 days/week with L1 orally 75 mg/kg/d in five children). Serum ferritin (SF) concentration was used as a marker for efficacy of iron-chelation therapy. Data on SF levels collected from all participants for a three-years period (January 2003-January 2006) were retrospectively analysed. SF concentration was measured every three-four months. SF levels less than 2500 mg/L (2500 mcg/L) indicated effective chelation therapy, whereas higher SF concentrations indicated ineffective treatment. Results. DFO alone was found ineffective in 9/80 patients (11.2%) mainly due to poor treatment compliance. Combination therapy demonstrated inefficacy in one child, 1/5. (20%). No adverse events associated with DFO or L1 were recorded. Conclusions. In patients with good treatment compliance, DFO is effective in reducing iron overload. For thalassemics with poor compliance, more flexible approach, including combination therapy, should be considered.

1146 UNRELATED STEM CELL TRANSPLANTATION FOR CHILDREN β-TALASSEMA MAJOR IN CHINA
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Backgrounds. Allo-genetic stem cell transplantation(SCT) is the only way to cure β-thalassemia major in present. But most children with β-thalassemia major have no chance of undergoing stem cells transplantation due to lack of matched sibling because of family policy in China. Aims. Unrelated SCT has been studied to Chinese children with β-thalassemia major to explore it’s method and effects in China. Methods. Eleven children of β-thalassemia major had undergone unrelated SCT between Jan. 2005 and Dec. 2005. Six recipient/donor pairs are 6/6 HLA(A,B,DRB1) high resolution typing matched and five recipient/donor pairs are 1/6 HLA high resolution typing mismatched. Six children received peripheral blood stem cells transplantation (PB SCT) and 6 children received bone marrow transplantation (BMT). β-thalassemia major children conditioned with BU 14-21 mg/kg+CY(140-200 mg/kg)+ ATG(25-35 mg/kg)+Flu(200 mg/m2). The graft contained median mononuclear cells 5.8x108/kg (range, 2.2x108/kg -10.0x108/kg) and median CD34+ cells 6.6x106/kg (range, 1.4x106/kg -22.5x106/kg). All patients received CSA FK506+MMF (MTX) as graft versus host disease (GVHD) prophylaxis and heparin + pGE1 as veno-occlusive disease (VOD) prophylaxis. Results. All of 11 children had got complete chimerism by the median day +11. No patient developed unusual organ toxicities including higher incidence of severe aGVHD in unrelated ACHIEVED BY IMATINIB ALONE IN CHRONIC MYELOCULEAR LEUKEMIA
P. Mishra, S. Sazawal, M. Mahapatra, R. Saxena, D.R. Choudhary, A. Dixit, T. Chatterjee, R. Kumar, V.P. Choudhary
AIMS, NEW DELHI, India

Backgrounds. The introduction of Imatinib, a targeted therapy for chronic myeloid leukemia (CML) has been a major advance in the treatment of chronic myeloid cancer management. However molecular techniques have shown that only 4-10% of Imatinib treated patients achieve complete molecular remission. Aim. To compare Imatinib alone with Imatinib-cytarabine to see if the combination could achieve greater molecular
responses. Methods. 85 newly diagnosed adult CML patients were ran- domized to receive Imatinib or Imatinib-cytarabine combination. Only those patients in chronic phase were included in the study. Hydrox yurea was the only therapy received by these patients prior to starting Imatinib. Imatinib was initiated at a dose of 400 milligrams, within 6 months of diagnosis. Cytarabine was added in 45 patients at a dose of 10 milligram /square meter for 10 days every month after 3 months of Imatinib. BCR-ABL transcripts were measured by real time PCR at baseline and at follow-up every 6 months. The reduction in BCR-ABL transcripts in the two groups was compared using Mann-Whitney test. Results. Patients were followed for a median period of 1.5 years (range 0.6-2.7 years) in the Imatinib group and 1.8 years (range 1-2.7 years) in the combination group. The baseline variables like age, Sokal risk groups and haemogram were similar in the two groups. Median values for age, haemoglobin, total leukocyte count and platelet count in the Imatinib group were 55 (range 21-45 years), 11gm% (range 6-15.5 gm%), 150000/cubic millimeter (range 112000-242000/cubic millimeter) and 540000/cubic millimeter (range 125000-1126000/cubic millimeter) respectively. Male: Female ratio was 5:1 in both the groups. No patient dropped out from the combination group. All patients achieved complete haematological responses. Both groups tolerated their therapies equally well with no significant difference in toxicity profiles. 2 patients in each group discontinued therapy because of grade 4 cytopenias. 1 patient in the Imatinib group and 2 patients in the combination group discontinued therapy because of grade 4 skin toxicities. The median number of BCR-ABL transcripts at diagnosis was 345808 (range 605-1012561), which had reduced to 18286 (range 0-5412661) at follow-up, a median log reduction of 1.255 (range 0.975-4.02). In the combination arm, the median number of BCR-ABL transcripts at diagnosis was 152496 (range 2713-1212285), which had reduced to 15415 (range 4-4532646) at follow-up, a median log reduction of 1.305 (range 0.12-8.2). This reduction in BCR-ABL transcripts in the two groups was not statistically significant (p=0.945). 2 patients in each group achieved more than 3 log reduction in BCR-ABL transcripts. Conclusion: We conclude that addition of cytarabine does not improve significantly upon the molecular responses seen with imatinib alone.

1149
CLINICAL SIGNIFICANCE OF QUANTITATIVE REAL-TIME PCR FOR MONITORING OF MINIMAL RESIDUAL DISEASE FOR PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE RECEIVING GLIVEC THERAPY

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RAMS, MOSCOW, Russian Federation

Background. With the high possibility of receiving major cytogenetic response (MCR) and complete cytogenetic response (CCR) for patients with chronic myeloid leukemia (CML) receiving Glivec therapy it is more significant to study minimal residual disease with the help of more sensitive methods than standart cytogenetic analyses. Real-time PCR is a specific and suitable method for quantitative characteristic of molecular response for CML patients treated with Glivec. Aim. Search of prognostically significant levels of minimal residual disease for CML patients in chronic phase (CP) treated with Glivec. Patients and methods. We have analysed 105 samples of peripheral blood and bone marrow and estimat ed a molecular response (MR) of 59 CML CP patients with MCR and CCR receiving Glivec therapy 400 mg daily after intervals with treatment failure. Median of observation was 36 months (6-54 months). We analysed levels of BCR-ABL™ 210 transcript by quantitative Real-time PCR, TaqMan technology (Applied Biosystems). Microglobuline was used as housekeeping gene. Results were expressed as ratio BCR-ABL/b2 microglobuline x10$. Results. In our investigation we observed a correlation between cytogenetic and molecular results, also a correlation between BCR-ABL transcripts levels for peripheral blood and bone marrow. In majority (52/ of 105) samples residual disease was detected by quantitative Real-time PCR. Decreasing of BCR-ABL transcript levels less than 2 log from baseline was associated with greater probability of cytogenetic relapse. 3 log and more decreasing of BCR-ABL transcript levels from baseline was associated with continuous MCR and CCR for all the patients. The patients with cytogenetic relapse had greater median of BCR-ABL transcript level than the patients with MCR and CCR. Cytogenetic relapse was preceded by increasing of BCR-ABL transcript levels. Conclusions. For the majority of CML CP patients treated by Glivec it was possible to detect minimal residual disease with the help of quantitative Real-time PCR. Probability of cytogenetic relapse depends upon BCR-ABL transcript level: less 2 log decreasing from baseline in our investigation predicted greater probability of cytogenetic relapse. Real-time PCR should be used as routine analyses for CML patients observation as routine analyses of minimal residual disease for CML patients.

1150
ACUTE HEPATITIS AFTER IMATINIB MESYLATE TREATMENT FOR CML: A CASE REPORT AND REVIEW OF THE LITERATURE

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Imatinib is a well tolerated oral anticancer drug. It is mainly metabolised by liver. Severe hepatic dysfunction occurs in fewer than 5% patients treated. We report a case of severe hepatitis documented by liver biopsy and we analysed similar cases reported in the literature. Case report. A 69-year-old female was diagnosed having CML in the chronic phase. Soon after diagnosis, on 11 August 2003 she started imatinib at a standard dose of 400 mg/d. Three months later she achieved a partial cytogenetic response in the bone marrow and she presented with a slight increase in aspartate aminotransferase (AST 68 U/L) and alanine aminotransferase (ALT 82 U/L). On December 2004 the AST was discontinued because of a progressive increase of hepatic enzymes (AST 158 U/L, ALT 267 U/L). The patient was well and asymptomatic. No other biochemical abnormality was observed. On January 14th, 2004 transaminases peaked at AST 403 U/L and ALT 797 U/L. Serologic tests for hepatitis A, B and C, for EBV, CMV and HSV were all negative. Ultra-sonography of the abdomen was normal. Six weeks after imatinib withdrawal we performed a percutaneous liver biopsy. Histological examination revealed a severe necrosis of hepatocytes with some grade of fibrosis and diffuse inflammatory infiltrates.

Table 1. Clinical and laboratory features.

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<th>Imatinib dose (mg/d)</th>
<th>Others</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>Rechallenge</th>
<th>Follow-up</th>
<th>Pharmacokinetics (Hepatic function)</th>
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$.$ time after discontinuation of imatinib and biopsy; &&: on imatinib treatment with prednisione and ursodeoxycholic acid

On March 2004 bone marrow aspiration documented lost of the cytogenetic response (100% Pb positive metaphases). Histological examination of liver one year after stopping imatinib was greatly ameliorated and showed minimal changes. We decided to reintroduce the drug (100 mg/day). Plasma levels of imatinib were measured by HPLC/MS on day 1, 5, 10 and 14 (baseline, half-an-hour, 1h, 2h, 4h and 8h after administration). The pharmacokinetics profile of imatinib was comparable to that obtained with standard dose in CML and GIST patients but on day 10 at steady state (SS), a marked increase of the main circulating metabo-
The tyrosine kinase inhibitor imatinib (STI571) is highly effective in the treatment of chronic myeloid leukemia (CML) patients. Deletions adjacent to the translocation junction on the derivative chromosome 9 were described in 90% of chronic myeloid leukemia (CML) patients. Deletions of chromosome 9 sequences located telomeric to the ABL gene apart from the BCR genes was performed using a pool of PAC, RP5-835J22 and RP5-1132H12, and the BAC RP11-164N13, respectively. A set of BAC/PAC clones (proximal and distal to ABL and BCR, respectively) belonging to some, a faint ABL signal on der(9) and a split BCR signal on der(6) and der(12) chromosomes. Reiterative FISH experiments using appropriate probes (proximal and distal to ABL and BCR, respectively) belonging to the UCSC database (http://www.genome.ucsc.edu) was queried for BAC/PAC probe locations and for gene identification. Results. Case #1. FISH experiment with BCR and ABL specific probes revealed one fusion signal on der(22) chromosome, a faint ABL signal on der(9) and a split BCR signal on der(6) and on der(12). Retargeting of the disease. Recent reports have identified the Src Kinase Hck to interact with BCR-ABL kinase possibly through the STAT5 pathways to induce cellular transformation. Aim. To investigate the role of Src Kinase Hck on BCR-ABL induced cell transformation as well as its importance in STI571 resistance. Materials and Methods. K562 Cells resistant to 5µM of STI571 were cloned and compared to non-STI571 resistant K562 as well as non-BCR-ABL expressing cells (KG-1). All cell lines were incubated with (5µM and 10µM STI571) alone or in combination with (5Nm and 10Nm The Src-Kinase inhibitor PP2). Viability studies were conducted using Trypan blue exclusion technique and DNA Fragmentation assays. Protein expression and tyrosine kinase activity were performed using Immunoprecipitation and Western Blotting techniques. Results. The combination of STI571 and PP2 have shown significant synergistic effect in reducing cell viability and tyrosine phosphorylation of both BCR-ABL and Hck in addition to downstream signalling proteins including JAK and STAT. This synergistic effect was observed even at lower concentration of STI571 (5 µM). Significantly, no effect was seen on non-BCR-ABL expressing cells. Conclusion. These results further support the importance of the Src Kinase Hck as a downstream signalling kinase for BCR-ABL and the possible role of this pathway in STI571 resistance.

NEW GENOMIC ISSUES ON DER(9) DELETIONS IN CHRONIC MYELOID LEUKEMIA

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1University of Bari, BARI, Italy; 2University of Foggia, FOGGIA, Italy.

Background. The Philadelphia (Ph) chromosome is found in more than 90% of chronic myeloid leukemia (CML) patients. Deletions adjacent to the translocation junction on the derivative chromosome 9 were described by several groups. These studies revealed two main points:1) genomic microdeletions were concomitant to the t(9;22) translocation; 2) deleted sequences were located upstream to ABL and downstream to BCR genes. We report a detailed molecular cytogenetic characterization of chromosomal rearrangements in two CML cases bearing deletions on der(9) without the characteristics reported above. Aims. We performed a molecular cytogenetic analysis by FISH to precisely characterize chromosomal events occurring in a case bearing a complex variant t(9;22) and in a case with ins(9;22)(q34;q11). Methods. Both patients were diagnosed and tested by conventional cytogenetic analysis, fluorescence in situ hybridization (FISH), and RT-PCR. FISH identification of the ABL and BCR genes was performed using a pool of PAC, RP5-835J22 and RP5-1132H12, and the BAC RP11-164N13, respectively. A set of BAC/PAC probes (proximal and distal to ABL and BCR, respectively) belonging to 9 and 22 chromosomes allowed us to define precisely the deletion size. The University of California, Santa Cruz (UCSC database (http://www.genome.ucsc.edu) was queried for BAC/PAC probe locations and for gene identification. Results. Case #1. FISH experiment with BCR and ABL specific probes revealed one fusion signal on der(22) chromosome, a faint ABL signal on der(9) and a split BCR signal on der(6) and on der(12). Retargeting of the disease. Recent reports have identified the Src Kinase Hck to interact with BCR-ABL kinase possibly through the STAT5 pathways to induce cellular transformation. Aim. To investigate the role of Src Kinase Hck on BCR-ABL induced cell transformation as well as its importance in STI571 resistance. Materials and Methods. K562 Cells resistant to 5µM of STI571 were cloned and compared to non-STI571 resistant K562 as well as non-BCR-ABL expressing cells (KG-1). All cell lines were incubated with (5µM and 10µM STI571) alone or in combination with (5Nm and 10Nm The Src-Kinase inhibitor PP2). Viability studies were conducted using Trypan blue exclusion technique and DNA Fragmentation assays. Protein expression and tyrosine kinase activity were performed using Immunoprecipitation and Western Blotting techniques. Results. The combination of STI571 and PP2 have shown significant synergistic effect in reducing cell viability and tyrosine phosphorylation of both BCR-ABL and Hck in addition to downstream signalling proteins including JAK and STAT. This synergistic effect was observed even at lower concentration of STI571 (5 µM). Significantly, no effect was seen on non-BCR-ABL expressing cells. Conclusion. These results further support the importance of the Src Kinase Hck as a downstream signalling kinase for BCR-ABL and the possible role of this pathway in STI571 resistance.

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MONITORING OF BLOOD-HISTAMINE LEVELS IN PATIENTS WITH CML DURING TREATMENT WITH IMATINIB

1Medical University of Vienna, VIENNA, Austria; 2St. Anna Children’s Hospital, VIENNA, Austria; 3Hospital Lainz, VIENNA, Austria

Backgrounds. The tyrosine kinase inhibitor imatinib (STI571) is highly effective in the treatment of chronic myeloid leukemia (CML). However, although most patients show a complete cytogenetic response (CCR) to imatinib, drug resistance may occur. Therefore, monitoring of minimal residual disease (MRD) during imatinib-therapy is a pivotal approach. Hence, most MRD parameters currently in use are expensive and require special technology and equipment. In this study, the value of whole blood histamine as a simple MRD marker of CML has been evaluated. Patients and Methods. Histamine levels were determined serially in whole blood samples before and during treatment with imatinib in 97 patients with CML by a specific radioimmunoassay. Results. Histamine levels were found to be highly upregulated in CML at diagnosis compared to healthy controls, and correlated with the presence of basophils. During treatment with imatinib, blood histamine levels decreased significantly in CML patients and returned to normal levels in those achieving a CCR. In all cases, loss of CCR during therapy was accompanied by an increase in histamine. Whereas the number of basophils were found to correlate well with histamine levels during treatment with imatinib, no correlation was found between histamine and Ph+ metaphases or between histamine and the percentage of BCR/ABL, suggesting that histamine is an independent MRD variable. Conclusion. Our data show that whole blood histamine levels are highly upregulated in patients with CML and should be considered as a simple reliable new marker to monitor MRD in patients with CML.
sibility of intensive chemotherapy in these patients. The aim of the study is to value the difference in EFS and OS among 2 groups of AML elderly patients treated with intensive chemotherapy (IC) or maintenance (M). From June 2001 to January 2006 we have treated in our Division 54 AML patients, 30 male and 24 female with median age of 73 years (66-90 years). 27 patients (16 M and 11 F with median age of 71 years) have received intensive chemotherapy (I.C. Flag and MICE) and 27 (14 M and 13 F with median age of 78.25 years) have received maintenance (low dose cytarabine and/or support). In IC group 12 patients (45%) have obtained to complete remission (CR) with to EFS and OS media of 4, 47 and 7, 15 months respectively, the rate of TRM has been of 25%. In the M group the CR has been documented in 8 patients (50%) with to EFS and OS of 4,22 and 4,94 months respectively (graph 1).

This results have shown a best rate of CR in the IC group but the OS and EFS difference is not statistically significant in the two groups ($p=0.7$). In conclusion the Intensive chemotherapy has not improved the survival in AML elderly patients. New therapeutic strategy is necessary for to improve the EFS and OS in these patients. Interesting is the use of specific monoclonal antibodies (anti CD55) in this poor disease especially in maintenance after a CR obtainable with an intensive or low dose chemotherapy.

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ACUTE MYELOID LEUKEMIA IN PATIENTS AGED 70 OR OLDER. EXPERIENCE AT A SINGLE CENTRE

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The management of old patients with acute myeloid leukemia remains controversial, specially in those cases that can be considered very old patients (aged 70 or older) in which the dilemma therapeutic abstention versus treatment (with low or high intensity) can be considered. We present our experience with this group of patients in the period 1990-2005. During the period of study 56 cases were diagnosed (EAP M3 cases were excluded). Patients were divided into 3 groups according to the treatment: no treatment, low intensity treatment (low doses Ara-C: 10 mg/m² s.c. days 1-21) and high intensity treatment (adapted ICE: Idarubicin 10 mg/m² days 1 and 5; Ara-C 100 mg/m²/12h days 1-3; Etoposide 100 mg/m² days 1-3). The mean age of patients was 76.29 years (70-85); sex distribution was 29 males and 27 females; mean Karnofsky index was 70.5 (30-100); 39 patients received treatment and 17 did not; overall survival was 5.8 months (median 2; 0.06-90+); almost significant differences were observed in the mean overall survival between the treated and no-treated groups (7.4 vs 2.1, months respectively, $p=0.06$). In the low intensity group (50 patients) an overall response of 55.3% (6 CR, 4 PR, 13 NR and 7 not evaluable) was observed while in the high intensity one (9 patients) this overall response was 44.4% (4 CR, 0 PR, 3 NR and 2 not evaluable); no statistical differences were observed between both groups ($p=0.16$). Considering overall survival in these same groups, no statistical differences were observed between them 7.2 (0.25-90+ vs 8.1 (0.5-28) months ($p=0.95$) respectively between the low and high intensity groups. Overall survival in the treated group is higher than in the non-treated one, differences almost reach statistical significance ($p=0.06$). Though no statistical differences have been found in the overall survival between both groups of treatment, this event could be explained by two reasons: the very long survival in one patient in the low intensity group and the still short follow-up of some patients in the high intensity one. Comparing both arms of treatment, a higher proportion of CR can be observed in the high intensity group (44.4% vs 20%, respectively), however, if this circumstance will contribute to a longer survival is still unknown.

1156

NGK2 RECEPTOR EXPRESSIONS IN UNTREATED ADULT PATIENTS WITH ACUTE MYELOGENOUS LEUKEMIA

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Backgrounds. Natural killer (NK) cell is one of the important cytotoxic lymphocytes for innate immune response to tumor cells as well as infected cells. The balance of the activating and inhibitory receptors of NK cells can determine the activity of cytotoxicity. Based on recent advances in the understanding of NK cells, baseline expressions of NGK2 might be involved in the immune response that regulate genome integrity in addition to their antigen-specific activation in a challenging clinical condition such as acute myelogenous leukemia (AML). Aims. In this study, we investigated expressions of C-type lectin-like receptors, i.e. CD94/NKG2A and NKG2D, in adult patients with AML. Together, it is expected that other researchers will also examine the role of ethnic differences in the phenomena described here. Methods. PB samples from 24 normal donors and 14 untreated AML patients were enrolled. Flow cytometric analysis using CD56, CD16, CD8, NK2G2A, and NKG2D-specific monoclonal antibodies were performed. Results. The proportion of CD16+CD3- NK cell and CD56+CD8- NK cell in peripheral lymphocytes were 3.48±3.80% vs 2.62±5.99% and 4.03±3.76% vs 8.61±8.70% in AML and control, respectively. NKG2D+ and CD94/NKG2A2 cells among CD56+CD8- fraction were 35.7±5.4% vs 24.4±5.9% ($p=0.13$) and 26.8±4.1% vs 37.7±3.7%, respectively. Therefore, NGK2D expression of NK cells in AML patients was statistically significantly decreased compare to control ($p=0.05$). Summary/Conclusions. While the expression of NKG2D, activating receptor of NK cell is relatively increased, the expression of CD94/NKG2A2, inhibitory receptor of NK cell is decreased in AML patients, which means some other mechanisms including altered responding ligand(s) are engaged.

1157

INCREASE IN SERUM SOLUBLE HLA-I IN ACUTE MYELOID LEUKEMIAS LEADS TO FAS LIGAND-MEDIATED APOPTOSIS OF CD8+ LYMPHOCYTES

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Soluble HLA-I (sHLA-I) molecules have been firstly described in the serum and urine of healthy individuals. More recently, it has been shown that the serum level of these soluble molecules is significantly increased in patients with an activation of their immune system, such as during allotraft rejection, acute graft versus host disease after bone marrow transplantation, autoimmune diseases or viral infections. Moreover, sHLA-I molecules can be released by tumor cells, and high sHLA-I serum levels have been found in solid cancers, melanomas and lymphomas. Thus, sHLA-I molecule are not specific markers for organ rejection, but rather are affected by inflammatory processes and viral or neoplastic transformation. In this study, we show that high serum levels of soluble HLA class I molecules (sHLA-I, range: 0.7-1.7 mg/mL) and soluble Fas ligand (Fasl, range: 0-4.1 mg/mL) are detected in patients with acute myeloid leukemia (AML) at diagnosis, compared to healthy donors (sHLA-I range: 0-1.0-0.6 mg/mL; sFasl range: 0.1-0.4 mg/mL). Both sHLA-I and sFasl serum concentrations increased during chemotherapy. The functional role of sHLA-I molecules either in physiological or in pathological conditions is not clear: it has been described that HLA-I molecules can trigger cytotoxic T lymphocytes to release cytokine and pro-inflammatory cytokines. However, we and others reported that sHLA-I molecules bind to CD8 receptors expressed on cytotoxic effector lymphocytes leading to activation-induced apoptosis or cell death mediated by synthesis and secretion of Fasl, and the consequent inter-
action with Fas expressed by T and NK cells. AML patients’ sera were able to induce transcription and secretion of FasL in CD8+ T cells, followed by apoptosis in vitro; this apoptosis was inhibited by either anti-HLA-1 or anti-Fasl specific monoclonal antibodies. These findings closely relate to the in vivo up-regulation of Fasl transcription observed in peripheral blood lymphocytes from AML patients; in the same cells, mRNA for the antipapoptotic protein Bcl-2 was down-regulated. Interestingly, caspase-8 and caspase-3, both downstream mediators of death receptor-induced apoptosis, were activated in vivo in CD8+ cells of AML patients, but not of healthy donors. These data strongly suggest that in AML, increased levels of sHLA-1 molecules may be responsible for the elimination of potentially anti-tumor effector cells through a Fas/Fas interaction.

### 1158

**THE NUMBER AND APOPTOSIS OF CIRCULATING ENDOTHELIAL CELLS IN THE PERIPHERAL BLOOD IS SIGNIFICANTLY INCREASED IN PATIENTS WITH ACUTE MYELOID LEUKEMIA AND REFRACTORY ANEMIA WITH EXCESS OF BLASTS**


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**Objectives.** The circulating endothelial cells (CEC) are proposed to be a noninvasive marker of angiogenesis. Material and Methods. We evaluated the absolute counts of CEC, their resting (rCEC) and activated (aCEC) subsets, circulating endothelial progenitor cells (CEPC) as well as apoptotic CEC (CECCAnnV+) in peripheral blood (PB) of 70 untreated patients with acute myeloid leukemia (AML) and 23 with myelodysplastic syndrome (MDS)/RAEB type (RAEB). The control group consisted of 90 healthy controls. CEC counts were evaluated by four-colour flow cytometry using a previously described panel of monoclonal antibodies. CEPC were defined as CD45-CD34-CD38- and CD133+. rCEC were defined as CD45-CD133-CD34+CD38+CD146+ and negative for activation markers (CD105, CD106). CD105 or CD106 positive mature endothelial cells were classified as aCEC. The levels of CEC were correlated with known prognostic factors. Additionally, apoptotic CEC were detected in PB using Annexin V assay (CD146+/Annexin-V+ cells, CEPCAnnV). The percentage of CEPCAnnV among the whole CEC number was determined. Results. There were highly significant differences in the count of CEC and their particular subtypes between AML and RAEB patients as well as the controls. The results (median counts and ranges) are presented in the Table. The positive correlation between CEC and CEPC counts was observed in both AML (r=0.45; p<0.001) and RAEB (r=0.64; p<0.01). The numbers of apoptotic CEC (CECCAnnV+) in both AML and RAEB were significantly higher than in the control (p<0.0001). However, in patients with RAEB the rate of CECCAnnV+ was significantly higher than in those with AML (p<0.0001). The number of microvascular origin CEC, depicted by CD36 expression, was also higher in MDS than in both AML (p<0.0001). Moreover, the negative correlation between CEC and absolute counts of white blood cells as well as PB blasts was observed in RAEB but not in AML. Conclusions. The CEC levels are significantly higher in AML and RAEB patients than in healthy subjects. These findings may suggest a relationship between clonal transformation and the substantially increased number of CEC. The rate of CECCAnnV+ is significantly elevated in AML and RAEB what may be due to increased turnover of CEC. Distinctly lower propensity of CEC to undergo apoptosis found in AML may correspond with more aggressive clinical course of this disease.

<table>
<thead>
<tr>
<th>Type of endothelial cells</th>
<th>AML n=70</th>
<th>MDS-RAEB n=23</th>
<th>Control group n=30</th>
<th>p value</th>
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</thead>
<tbody>
<tr>
<td>CEC (µL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(39-291,3)</td>
<td>27,2</td>
<td>12,5</td>
<td>2,95</td>
<td>a vs. c p&lt;0.0001</td>
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<td>(3,9-34,7)</td>
<td>(4,3-9,7)</td>
<td>(5,0-13,1)</td>
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<td>b vs. c p=0.0001</td>
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<tr>
<td>a CEC (µL)</td>
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<td></td>
</tr>
<tr>
<td>(9-87,7)</td>
<td>11,35</td>
<td>5,4</td>
<td>0,9</td>
<td>a vs. b p=0.01</td>
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<tr>
<td>(2,9-1,6)</td>
<td>(1,3-3,1)</td>
<td>(5,2)</td>
<td></td>
<td>b vs. c p=0.01</td>
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<tr>
<td>r CEC (µL)</td>
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<td></td>
</tr>
<tr>
<td>(203,6)</td>
<td>11,85</td>
<td>7,8</td>
<td>1,6</td>
<td>a vs. c p&lt;0.0001</td>
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<td>(0,4-10,8)</td>
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<td>CEPC (µL)</td>
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<tr>
<td>(40,2)</td>
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<td>1,9</td>
<td>0,1</td>
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<td>(0,1-1,2)</td>
<td></td>
<td>b vs. c p&lt;0.0001</td>
</tr>
</tbody>
</table>

### 1159

**AN ANTecedent DIAGNOSIS OF REFRactory ANEMIA WITH BLAST EXCESS HAS NO PROGNOSTIC RELEVANCE IN ACUTE MYELOID LEUKEMIA OF THE ELDERLY TREATED WITH AGGRESSIVE CHEMOTHERAPY**


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**Background.** Host related factors and disease related factors account for the unsatisfactory outcome of acute myeloid leukemia (AML) in the elderly. However, the exclusion in many trials of patients with previously diagnosed myelodysplastic syndromes (MDS) renders uncertain the evaluation of the prognostic relevance of secondary AML (s-AML), defined as AML arising after either a history of chemotherapy or radiotherapy for a previous malignancy or a preceding history of MDS or hematologic malignancies. **Aims.** To evaluate the prognostic relevance of a previous diagnosis of refractory anemia with excess of blasts (RAEB) in terms of complete remission (CR) achievement and duration, survival and feasibility of autologous stem cell transplantation (ASCT) in elderly patients with AML.

**Patients and methods.** Among 166 consecutive elderly AML patients observed in the period 2001-2005, 87 cases (median age: 69 years, range 61-81) were enrolled into an aggressive chemotherapy program consisting of a combination of fludarabine and a short course of busulfan, followed by autologous bone marrow transplantation (ABMT) or ASCT in first complete remission (CR). AML patients were classified as s-AML if a previous diagnosis of RAEB was documented in parallel with a blastic transformation of AML. The control group consisted of 80 patients with de novo AML.

**Results.** There were highly significant differences in the number of CEC and their particular subtypes between AML and RAEB patients as well as the controls. The results (median counts and ranges) are presented in the Table. The positive correlation between CEC and CEPC counts was observed in both AML (r=0.45; p<0.001) and RAEB (r=0.64; p<0.01). The numbers of apoptotic CEC (CECCAnnV+) in both AML and RAEB were significantly higher than in the control (p<0.0001). However, in patients with RAEB the rate of CECCAnnV+ was significantly higher than in those with AML (p<0.0001). The number of microvascular origin CEC, depicted by CD36 expression, was also higher in MDS than in both AML (p<0.0001). Moreover, the negative correlation between CEC and absolute counts of white blood cells as well as PB blasts was observed in RAEB but not in AML. Conclusions. The CEC levels are significantly higher in AML and RAEB patients than in healthy subjects. These findings may suggest a relationship between clonal transformation and the substantially increased number of CEC. The rate of CECCAnnV+ is significantly elevated in AML and RAEB what may be due to increased turnover of CEC. Distinctly lower propensity of CEC to undergo apoptosis found in AML may correspond with more aggressive clinical course of this disease.
CD56 ANTIGEN EXPRESSION IN ACUTE MYELOID LEUKEMIA; CLINICAL AND IMMUNOPHENOTYPIC IMPLICATIONS

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Background and Aims. The CD56 antigen expression has been reported in several hematologic malignancies; it is found in 10-30% of cases of acute myeloid leukemia (AML). Its prognostic impact remains uncertain although it has been associated with an unfavorable outcome, especially in AML M2 and M4. Recently, two novel CD56+ malignancies (the CD7/CD65 myeloid/NK and the CD4/CD56+ dendritic cell leukemias) were described. We investigated the immunophenotypic identity of the CD56+ AML and its correlations with other disease characteristics. Patients and Methods. From 1999 to 2005, 127 samples of fresh bone marrow or peripheral blood of AML patients (M0: 65/62, median age: 59 (11-85) years), were analyzed in our laboratory. One-, two- or three-color flow cytometry was performed on Coulter Epics cytometer. Positivity for surface (s) antigens expression was set at 20% and for cytoplastic (c) antigens at 10% of blasts stained with specific monoclonal antibodies for glycophorin A, CD2, sCD3, CD4, CD7, CD8, CD14, CD19, CD20, CD33, CD34, CD45, CD56, CD117, HLA-DR, MPO, lysozyme, lactoferrin, CD79a, CD80, CD123, CD13 and TdT. Results. Patients (pts) were classified according to FAB criteria as M0:10 pts, M1/2:51, M3:20, M5:12, M6:1 and M7:2 pts. 15.7% of the pts suffered from secondary leukemia. CD56 expression was detected in 29% (35/127) of the pts; this was higher in M1/2 (33%) and M5 subtypes (52%). CD56 positivity was not influenced by age, sex, WBC, blast percentage, Hb and platelet count at diagnosis, LDH, secondary leukemia and extramedullary disease. Statistical analysis revealed a positive correlation between CD56 expression and expression of CD34 (p=0.001, r=0.329), CD29 (p=0.014, r=0.221), CD8 (p=0.047, r=0.180), CD4 (p=0.006, r=0.249) and CD9a (p=0.017, r=0.281) antigens, while a negative correlation was detected for CD13 (p=0.038, r=0.187) and CD13 (p=0.081, r=0.163) expression. CD56+ AML group expressed also CD38 (87% of the pts), lysozyme (79%), HLA-DR (78%), CD13 (71.4%), MPO (69%) and CD34 (67%). The comparison between the CD56+ AML group and the CD56- leukaemias showed differences in the expression of lysozyme (79% of CD56+ AML vs. 54%, p=0.044), CD22 (6% vs. 0%, p=0.03), CD34 (67% vs. 92%, p=0.002) and CD13 (55% vs. 71%, p=0.066). Conclusions. The immunophenotype of CD56+ AML is characterized by the commitment to the myeloid/monocytic lineage (CD38, lysozyme, CD13, HLA-DR and MPO). The negative correlation between CD56 expression and CD13 and CD13 is a novel finding not previously reported. The further investigation of the possible relationship of the CD56+ AML with the NK/dendritic cell leukemias might be the key to explain its clinical behavior.

DISEMINATION OF HUMAN ACUTE MYELOID LEUKEMIA CELLS IN PRIMARY CULTURE IN RESPONSE TO DERIVATIVES OF METHYL ASIOMATE, PLANT STRESS HORMONE

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Backgrounds. Since several plant hormones and their analogues induce cell cycle arrest and inhibit human cell proliferation, these compounds may be therapeutic agents against human malignancies. Some regulators of plant growth and differentiation have been shown to induce the differentiation, since PD98059, an inhibitor of MAPK kinase, suppressed MAPK activation and induced myelocytic differentiation. MAPK activation was necessary for MJ-induced monocytic differentiation. MAPK activation was necessary for MJ-induced monocytic differentiation. MAPK activation was necessary for MJ-induced monocytic differentiation.

1163 DIFFERENTIATION OF HUMAN ACUTE MYELOID LEUKEMIA CELLS IN PRIMARY CULTURE IN RESPONSE TO DERIVATIVES OF METHYL ASIOMATE, PLANT STRESS HORMONE

Results. MJ-induced monocytic and granulocytic differentiation of HL-60 cells. MJ activated mitogen-activated protein kinase (MAPK) in the cells before causing myelomonocytic differentiation. MAPK activation was necessary for MJ-induced myelomonocytic differentiation. MAPK activation was necessary for MJ-induced myelomonocytic differentiation. MAPK activation was necessary for MJ-induced myelomonocytic differentiation.
of established cell lines, they may have only modest differentiation-inducing activity in freshly isolated leukemic cells. Therefore, we sought to determine whether the potent derivative of MJ could affect the differentiation of leukemic cells from patients with AML. In the present study, we examined the effect of MJ derivative on the differentiation of AML cells in primary culture and compared this differentiation-inducing activity with those of the well-known inducers all-trans retinoic acid and 1α,25-dihydroxyvitamin D3. Paxalone derivatives significantly stimulated both functional and morphological differentiation of leukemia cells in some AML cases. This differentiation-inducing activity was more potent than those of all-trans retinoic acid and 1α,25-dihydroxyvitamin D3. Paxalone derivatives significantly stimulated both functional and morphological differentiation of leukemia cells in some AML cases. This differentiation-inducing activity was more potent than those of all-trans retinoic acid and 1α,25-dihydroxyvitamin D3. Conclusion: Our final goal is perform clinical trials of paxalone derivatives in differentiation therapy for hematopoietic malignancies, either alone or in combination with other differentiation inducers so that their doses can be reduced. Paxalone and its analogs are used as flavorings in foods and in cosmetics, and MJ was therapeutically effective in an animal model of lymphoma, suggesting that these compounds may be clinically useful. One novel derivative is a particularly promising therapeutic agent for the treatment of leukemia.

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FLAG-(IDA)-MYELOTARG IN THE THERAPY OF RELAPSED AND REFRACTORY ACUTE MYELOID LEUKEMIA. PRELIMINARY RESULTS

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Background. Patients with relapsed and refractory AML have a bad prognosis. In this setting is often difficult to obtain a prolonged complete remission. In the last years Fludarabine and high dose Citarabine with or without Idarubicin and G-CSF have been frequently used (FLAG and IDA schedules). The immunotoxin gemtuzumab ozogamicin (Mylotarg(GO)) is a humanized IgG4 monoclonal antibody directed against the CD33 epitope, which is chemically linked to calicheamicin, a highly potent antitumor antibiotic. As a single agent it has been shown to be an effective agent in the treatment of relapsed AML with a tolerable toxicity profile. Recently the United Kingdom Medical Research Council (MRC) published the preliminary results of AML15 trial, which was designed to evaluate the effect of adding GO to each course of intensive consolidation chemotherapy in patients younger than 60 years as first-line treatment. These results are encouraging. Aims. We are using this association in the treatment of relapsed and refractory adult AML. Methods. Until now 18 patients have been enrolled in this trial and we show our preliminary results. 5 patients were resistant to induction; 8 patient were relapsed after conventional chemotherapy; 6 of them were in the first relapse, 1 was in the second and 1 in the third; 2 were relapsed after autologous transplant and 3 were relapsed after alloengenic transplantion (1 familiar and 2 unrelated). The median age was 54 years (range 20-74). The median time to the first relapse was 12 months. 9 of 20 patients were treated with Fludarabine 30 mg/m²/2-6, Citarabine 2.0 gr/m²/2-6, Idarubicine 10 mg/m²/4,5,6, Mylotarg 3 mg/m²/1, G-CSF 375 mg/dl 1-6. 2 patients with the same schedule without Idarubicine. Results. 12 patients obtained Complete Remission (70%), 2 died after therapy and 5 were resistant to this schedule. The last patients is not yet valutable. Out of the 12 patients in CR, 8 are in continuous complete remission (median 6 months, range 2-18). 6 of these underwent alloengenic BMT (2 familiar and 4 unrelated) and 3 are actually in CR, 1 died for progressive leukaemia, 1 relapsed and 1 is not yet valutable. Two are waiting for alloengenic transplant. Two patients relapsed at 3 and 4 months and died for progressive leukaemia. Conclusion. FLAG-IDG schedule is in our experience a feasible therapy for relapsed and refractory adult acute leukemia. The toxicity is acceptable. Complete Remission was obtained in 70% of patients with a single cycle. Many of these patients underwent to alloengenic transplant with acceptable toxicity.

1165

FLAG-IDG IN THE TREATMENT OF REFRACTORY/RELAPSED ADULT ACUTE MYELOID LEUKEMIA


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Background. Although several different chemotherapy combinations have been administered to patients with refractory/relapsed acute myeloid leukemia (AML), the prognosis in this subset of patients is still poor, with a complete remission (CR) rate ranging from 30 to 40%. The goal of induction chemotherapy varies from achievement of long-term CR to providing a bridge to hematopoietic stem cell transplantation (HSCT) aimed at prolonging disease-free (DFS) and overall survival (OS). Aims. In this study we evaluated the efficacy and the toxicity profiles of the combination of flavopiridol (FP), high-dose cytarabine (Ara-C), idarubicin and G-CSF in refractory/relapsed AML patients. Methods and patients. Between October 1998 and October 2005, 74 AML patients (35 M and 39 F, median age 43 years, range 15-60) were treated with FLAG-IDG (fludarabine 30 mg/m², Ara-C: 2 gr/m² for 5 days, idarubicin 10 mg/m² for 3 days and G-CSF 5 mg/kg from day +6 until neutrophil recovery). All patients underwent a cytogenetic evaluation; 4 (5.4%) were in the favourable-risk group, 30 (40.6%) in the intermediate-risk group, 31 (41.8%) in the poor-risk group and in 9 (12.2%) the karyotype was not available. Fifty-four patients (72.9%) were in first relapse; 44 after only chemotherapy, 7 after chemotherapy and autologous peripheral stem cell transplantation and 3 after chemotherapy and allogeneic peripheral stem cell transplantation. Twenty patients (27.1%) were refractory to conventional chemotherapy including cytarabine, etoposide and daunorubicin. Results. The overall CR rate was 48.6% (36 of 74); 28 of 54 (51.8%) in relapsed and 8 of 20 (40%) in refractory patients. There were 5/47 deaths (6.7%), 1 due to fungemia (C. tropicalis), 2 to sepsis (P. aeruginosa) and 2 to cerebral hemorrhage. The cumulative incidence of DFS and OS was 25% at 17 months and 31 months respectively; the 21 patients who received allogeneic stem cell transplantation (11 from a matched donor, 5 from a mismatched donor and 5 from an unrelated donor) and 7 patients received autologuous stem cell transplantation; 6 patients were judged unable to receive any further therapy and 5 refused other therapy. In the 36 responders, the disease-free survival (DFS) and overall survival (OS) were 10 (range: 6 - 22) and 12 (range: 6 - 29) months respectively; the 21 patients who received allogeneic stem cell transplantation had a DFS of 18 (range 4 - 68) months. Conclusions. In our experience, FLAG-IDG is a well-tolerated regimen in refractory/relapsed AML patients; the toxicity is acceptable, enabling most patients to receive further treatment, including transplantation procedures.

1166

NON-ADHERENT LEUKEMIA BLASTS CONVERT INTO ADHERENT FIBROBLASTS WITH THE CHARACTERISTICS OF ORIGINAL LEUKEMIA BLASTS

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Background and Aims. The cancer stem cell hypothesis has advanced, and in several malignancies including leukemia cancer stem cells have been identified, which behave similarly to normal stem cells in terms of their self-renewal and self conversion into differentiated cells with regard to their appearance, expression of cell-surface molecules and ability to make an exact recapitulation of the original heterogeneous malignant cells. We estimated whether non-adherent leukemia blast cells convert into adherent fibroblasts with a long-term liquid culture system. Method. From informed patients with acute leukemia or CML, bone marrow cells or the blood were collected. With gradient sedimentation method three mononuclear cell-fractions were prepared, which were further cultured. Fibroblasts were treated with Trypsin, harvested and further cultured. When leukemia blasts with fusion product of MLL and ELL, of MYH11 and CBF_ and of BCR and ABL were cultured for a long-term (>1 year), 20% of the cells changed their appearance, expression of cell-surface molecules and ability to self-renewal and self conversion into differentiated cells with regard to the similar characteristics to those of original leukemia blasts. The similar expression of cell-surface molecules was also observed to those of original leukemia blasts. (HSCT) aimed at prolonging disease-free (DFS) and overall survival (OS).
blasts. The generated fibroblasts had the same levels of functions to leukemia blast-derived fibroblasts, original leukemia blasts proliferated extensively. The generated fibroblasts expressed CD133, which is one of the important markers for cancer stem cells. Conclusion. These results indicate that leukemia blasts can create their own environment for proliferation.

1167 PREVALENCE AND PROGNOSTIC SIGNIFICANCE OF FLT3 MUTATIONS IN ACUTE MYELOID LEUKEMIA: ASSOCIATION OF ITDS WITH POOR OUTCOME IN PATIENTS WITH NORMAL CYTOGENETICS

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Backgrounds. Acute myeloid leukemia (AML) is a difficult disease to treat. Molecularly targeted therapy represents a novel therapeutic approach. Activating mutations of FMS-like tyrosine kinase 3 (FLT3) are present in approximately one third of de novo AML and have been implicated in its pathogenesis. The leukemic blasts of most AML patients have the internal tandem duplications (ITDs) in the juxtamembrane region or point mutations in Asp835 and Iso836 codons in the activation loop of the kinase domain (TKD) of the FLT3 receptor. Both mutations result in constitutive FLT3 receptor activity and may play a significant role in leukemogenesis. Aims. In this study we have analyzed the incidence and type of FLT3 mutations in a large series of newly diagnosed AML patients. Furthermore, we have evaluated the prognostic impact of FLT3 mutations. Methods. FLT3/ITD was determined by polymerase chain reaction (PCR). FLT3/ITD was associated with leukocytosis (106.8 x 10^9/L vs 30 x 10^9/L in FLT3-wt, p=0.015) and high percentage of circulating blast cells (82% vs 42% in FLT3-wt, p<0.0001). Differences, FLT3/ITD mutations were not associated with high white blood cells count and blast cells percentage. FLT3 mutations were more prevalent in patients with normal karyotype (51%). In this group, DFS and OS were significantly inferior for patients with FLT3/ITD than patients without mutations (0 vs 5, p=0.0032; 5 vs 9, p=0.049, respectively). Conclusions. We have identified the FLT3/ITD as an independent poor prognostic factor in patients with normal cytogenetics. Therefore, targeting FLT3 mutations represents a potential therapeutic target for AML. These results suggest that new treatment modalities, such as therapy with a FLT3 tyrosine kinase inhibitor, are clearly needed for this group of patients with ‘standard risk’ profile.

1168 IMPACT OF CHEMOTHERAPY TREATMENT IN AML PATIENTS AGED MORE THAN 60 YEARS OLD. A SINGLE CENTRE EXPERIENCE

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Institut Català d’Oncologia, L’HOSPITALET DE LLOBREGAT, Spain

Introduction: Overall prognosis in elderly patients with acute myeloid leukaemia (AML) is very poor. Nowadays patients older than 60 years who receive conventional chemotherapy achieve complete remission (CR) (27%) or AML patients with normal cytogenetics. Therefore, targeting FLT3 mutations represents a potential therapeutic target for AML. These results suggest that new treatment modalities, such as therapy with a FLT3 tyrosine kinase inhibitor, are clearly needed for this group of patients with ‘standard risk’ profile.
After a median follow-up of 17 months, 30% and 38% of the patients are in continuous CR in FLAI and ICE arm respectively. Our prospective randomised study confirmed the anti-leukaemic effect and the low toxic profile of FLAI as induction treatment for newly diagnosed AML patients.


### 1172

**ALLOGENIC STEM CELL TRANSPLANTATION WITH CD34+ SELECTED GRAFTS IN ACUTE MYELOID LEUKAEMIA A LONG-TERM FOLLOW UP**


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GVHD is a major cause of morbidity and mortality after ASCT. In order to decrease transplant related toxicity, in 1996 we have launched a program of ASCT with ex vivo CD34+ selected grafts in AML. Between August 1996 and July 2004, allogeneic stem cell transplantation with CD34+ positive selected PBSC has been performed in 57 patients with acute myeloid leukemia. In 6 pt the cytogenetics was of poor prognosis. In all cases the donors were matched HLA siblings. Median age: 36 years (5-55); sex: 15/22 (males/females). Status of disease at transplantation: 1st CR: 32; 2nd CR: 4; early relapse: 1. Myeloablative conditioning regimens used were: busulfan + cyclophosphamide + ATG (BuCy); 26; busulfan + fludarabine (FluBu): 11; After July 2002 lymphocytes were added back to infuse a target number of 0.3 x 10^6 CD3+ cells/kg recipient. GVHD prophylaxis was: cyclosporine+methotrexate: 20; cyclosporine only: 17. Median follow-up of surviving patients is 1813 days (range: 560 - 2942). Cells infused=10^6/kg (median (range)): CD34+: 5.1x10^6 (0.9 - 15.1); CD3: 0.19 (0 - 0.66). Thirty six evaluable patients engrafted. Five patients had late graft failure (median 354 days, range 113-1204).

### Table 1.

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<th>1 year</th>
<th>3 years</th>
<th>5 years</th>
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<tr>
<td>OS</td>
<td>83.8±6.1%</td>
<td>80.6±7.7%</td>
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<tr>
<td>DFS</td>
<td>70.3±7.5%</td>
<td>61.6±8.1%</td>
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There was no significant correlation between graft failure and the number of CD34+ or CD3+ infused. Probability of graft failure at one year was 18.2 ± 11.8% after FluBu and 4.6 ± 6.6% after BuCy (difference not statistically significant). Acute GVHD grade ≥ 2 occurred only in 2 pt. Two of 5 pt with graft failure are alive 3 years after a 2nd graft, in CR and with chronic GVHD. Other 6 patients had chronic GVHD: limited in 3 pt and extensive in 5 pt. Overall survival (OS), disease free survival (DFS) and relapse rate are shown in the table. Overall survival was significantly better if the number of CD3+ cells was < 0.19x10^6/kg; the OS was 94.4 (+5.4%), 88.9 (+7.4) and 83 (+9)% at 1, 3 and 5 years, respectively, when CD3+ infused < 0.19x10^6/kg and 72.2 (+10.6), 55.6 (+11.7) and 55.6 (+11.7)% when CD3 cells infused were > 0.19x10^6/kg. There was no significant difference in DFS between subgroups. Transplant related mortality at 100 days and at 1 year was 2.7 (+2.7) and 10.1 (+5.1)% respectively. In conclusion, in our experience ASCT with CD34+ selected grafts in pt wit AML is associated with a low risk of acute and chronic GVHD and seems to reduce transplant related mortality without an increase in relapse risk. These results confirm other studies of ASCT with lymphocyte depleted grafts in AML.

### 1173

**PU.1 AND C/EBPα EXPRESSION IN ACUTE MYELOID LEUKEMIA**

A. Di Ruscio, F. D’Alò, F. Guidi, E. Fabiani, G. Leone, M.T. Voso

*Catholic University, ROME, Italy*

**Backgrounds.** The myeloid transcription factors C/EBPα and PU.1 play a pivotal role in normal hematopoiesis. PU.1 has been shown to be essential for monocytic development, while C/EBPα is necessary for granulocytic differentiation of myelomonocytic precursors. In particular, their reciprocal expression is essential for lineage differentiation. Alterations

### FREDERICH'S FEVER IN A YOUNG INFANT:

**ARTHRITIS AS A COMPLICATION OF PRIMARY HERPES SIMPLEX INFECTION**


*National Medical Centre, Budapest, Hungary*

Acquired activating mutations of both fms-like tyrosine kinase 3 (FLT3) and Janus kinase 2 (JAK2) confer proliferative and survival advantage for leukemic cell clones. In this study we determined the incidence of FLT3-ITD and JAK2-V617F mutations in a cohort of 51 patients with M2 differentiation. Both mutations were detected in 2.2% (1/45). FLT3-ITD was positive in 2.2% (3/132) of AML patients. JAK2-V617F was positive in 2.2% (3/132). Three patients (2.2%) carried both mutations. FLT3-V617F mutation was positive in 2.2% (5/237) of AML patients. According to the prognostic significance of FLT3 mutations, only patients under 60 years of age receiving curative treatment were included. FLT3-ITD mutation was a negative prognostic factor. In the present study, 132 consecutive adults with newly diagnosed AML (57 males and 75 females) treated in our institute between January 2001 and December 2005 were enrolled. The median age of onset was 49 ± 14 (range 18-83) years. We analysed FLT3-ITD and JAK2-V617F mutations by fluorescent PCR and PCR-RFLP methods; and JAK2-V617F by allelic-specific PCR at the time of diagnosis. FLT3 was present in 25.5% (31/123) of the patients. JAK2-V617F mutations were detected in 6.8% (9/132). Three patients (2.2%) carried both mutations.

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### 1173

**PU.1 AND C/EBPα EXPRESSION IN ACUTE MYELOID LEUKEMIA**

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*Catholic University, ROME, Italy*

**Backgrounds.** The myeloid transcription factors C/EBPα and PU.1 play a pivotal role in normal hematopoiesis. PU.1 has been shown to be essential for monocytic development, while C/EBPα is necessary for granulocytic differentiation of myelomonocytic precursors. In particular, their reciprocal expression is essential for lineage differentiation. Alterations
We have investigated C/EBPα and PU.1 expression levels, and their reciprocal ratio, in different subsets of AML and correlated these data to morphology, FLT3-ITD mutations and cytogenetics. Bone marrow mononuclear cells (BMMC) were isolated from 117 patients with AML at the time of diagnosis and from 13 normal bone marrows using Ficoll gradient. CD34+ cells were isolated from normal bone marrow by immunomagnetic separation. Granulocytes, monocytes and lymphocytes were isolated from buffy coat of normal donor. AML diagnosis was made according to WHO criteria. Cytogenetic data were available for 73 patients. C/EBPα and PU.1 levels were quantified by real time RT-PCR using 18S as reference gene. FLT3-ITD mutation was studied using current protocols. Results. Heterogeneous expression of PU.1 and C/EBPα was observed in different AML subsets. Higher levels of PU.1 and C/EBPα were observed in promyelocytic and myelomonoblastic leukaemias when compared to normal BMMC (p<0.02 for both). Since most AML patients started ATRA before doing bone marrow aspirate, drug-induced up-regulation of PU.1 could be observed in these patients’ samples as recent reports described. On the other side, lowest PU.1 levels were observed in acute erythroid leukaemias and, when compared to normal BMMC, this difference was statistically significant (0.09 vs 4.12, p<0.05). Down-regulation of C/EBPα was observed in AMLs with t(8;21) and acute erythroid leukaemias. We observed that PU.1/C/EBPα ratio was higher in monocytes and decreased progressively from peripheral granulocytes to CD34+ cells. When analysing the distribution of PU.1/C/EBPα ratio, AMLs with t(8;21) showed the highest ratio (median ratio=4.2), while acute erythroid leukaemias had the lowest ratio (median ratio=1.4). In particular, when comparing AMLs and normal BMMC, the differences in PU.1/C/EBPα ratio reached statistical significance (p<0.0001 and p=0.05, respectively). Since down-regulation of C/EBPα and PU.1 has been described in cell lines expressing FLT3-ITD, we correlated their expression to FLT3 mutations. FLT3-ITD were present in 18 of 112 patients studied (16%) but no differences were observed in PU.1 and C/EBPα levels in mutated and unmaturated patients. Moreover, to verify the functional importance of these data, we studied the expression of two C/EBPα and PU.1 target genes, G-CSFR and M-CSFR respectively, in patients with high and low levels of these transcription factors. We found a direct correlation between levels of PU.1 and M-CSFR and between levels of C/EBPα and G-CSFR. Summary/Conclusions. C/EBPα and PU.1 expression and alteration of their reciprocal ratio may play a role in the pathogenesis of specific subsets of AMLs. Deregulated expression of these transcription factors may lead to an ineffective transcriptional control of hematopoiesis.

1174
HEART INFARCT AS THE MAJOR CAUSE OF EARLY DEATH OF HEMATOLOGICAL PATIENTS AS IDENTIFIED BY AUTOPSY
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While majority of hematological patients die due to either their disease or to adverse reactions to their treatment, there is a paucity of studies that use autopsy to more precisely identify the actual causes of death in each case and to relate this to the clinical diagnosis. More precise knowledge of such causes in hematological malignancies and aplastic anaemia would allow to properly focus research efforts and possibly to decrease mortality rates. In this study, the results of 154 autopsies of patients (the largest such series in the literature) with hematological diseases were compared with clinical data. They concerned 13.6% of 1129 patients who died in this Department in the years 1996-2005. The most probable causes of death in particular hematological diseases, discordancies between clinical and autopsy diagnoses, and their relation to clinical characteristic were identified in the studied cohort of patients. Down-regulation of C/EBPα was observed in AML at the time of diagnosis, but this down-regulation at this time was not explained by the clinical course and in 50% was sudden. Although various infections combined have been found to be responsible for the largest number of deaths (26.6%), the most common single cause was myocardial infarction (29 patients or 18.8%). Moreover, the myocardial infarction was found to be the most common cause of death in all age groups (18–42; 45–62; 60–90 years) and in majority of hematological malignancies (acute myeloblastic leukaemia, acute lymphoblastic leukaemia, non-Hodgkin’s lymphoma, Hodgkin’s lymphoma, multiple myeloma, chronic lymphocytic leukaemia). Furthermore, this fatal myocardial infarction frequently occurred early after diagnosis and initiation of treatment, and not in the course of a relapse of the disease. The discordance between clinical and autopsy diagnosis of immediate cause of death was found in 55 patients (55.7%, 95% c.i. 28.2–42.8%) of which 59.0% of cases were considered class I discrepancy according to Goldman’s criteria. The myocardial infarction was found to be clinically undiagnosed in 69% of cases. In 41% it was class I discrepant diagnosis. These data suggest that hematological patients require special attention and probably preventive measures concerning myocardial infarction particularly during initiation of antineoplastic therapy.

1175
HUMAN CD34 POSITIVE RESISTANT MYELOID LEUKEMIA CELLS EXPRESS THE EMBRYONIC STEM CELL ANTIGENS: OCT-4 AND CD133
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Lancashire School Postgraduate Medicine, PRESTON, United Kingdom

In 1942, Globus & Kuklenbeck proposed the presence of embryonic remnants in the sub-ventricular zone of human brain capable of giving rise to malignant tumours [Arch Pathology, 34, 674-734]. Recently small population of OCT-4 positive embryonic stem cells has been identified in adult murine bone marrow. Also, human CD133 positive cord blood stem cells co-express OCT-4 and other embryonic genes. The aim of the present study was to investigate the presence of embryonic stem cell markers in human AML CD34 positive cells.

We examined the presence of the two isoforms of human stem cell CD133 antigen and the embryonic OCT-4 antigen in KG1a human CD34 positive resistant AML cells. Both immunofluorescence and immunocytochemical stainings of AML CD34 positive resistant cells with anti-CD133 epitope-2 (clone AC133, Miltenyi Biotec Ltd) and anti-OCT-4 (clone sc-5279, Santa Cruz Biotechnology Inc.) revealed the presence of OCT-4 and CD133 epitope-2 antigens but not CD133 epitope-1 antigen. More than 90% of KG1a AML cells were OCT-4 positive in three experiments using both negative and positive controls. OCT-4 positive cells have significantly larger size than negative cells. The presence of CD133 epitope-2 and not epitope-1 in these AML CD34 positive cells is in line with the CD133 epitope expression in normal endothelial and haematopoietic stem cells. The expression of OCT-4 embryonic antigen in both normal bone marrow and leukaemia cells provide new support for the 60-year old hypothesis of ‘embryonic remnants’ in adult life being target for and capable of malignant transformation. Further studies are warranted to evaluate the presence of embryonic stem cell antigens in AML blast cells from patients and their functional relevance to resistance to chemotherapy and any prognostic value.

1176
LEUKEMIC INFILTRATION OF THE RETINA AT ONSET OF PHILADELPHIA POSITIVE ACUTE LYMPHOPROLIFERATIVE LEUKEMIA REVEALED BY STRATUS OPTICAL COHERENCE TOMOGRAPHY (OCT)
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University Hospital, UDINE, Italy

A 55-year-old man was admitted with pancytopenia and a loss of vision with a central visual field defect in his left eye. Bone marrow evaluation revealed acute lymphoblastic leukaemia, B-lineage phenotype, cytogenetic and molecular genetic analysis showed t(9;22) and a P210 positivity (BCR-ABL, a2b2). The cerebrospinal fluid was positive for leukaemic cells (100 blast cells/µL). Ophthalmic examination was performed. Fundoscopy showed a lifting and detachment of neuroretinal epithelium in the left eye, which was confirmed by ultrasound examination (vitreous cavity was normal). Stratus Optical Coherence Tomography (OCT) showed a retinal detachment with a choroidal infiltrate in the left eye (Figure 1). Brain and orbital magnetic resonance imaging were normal. The patient underwent induction chemotherapy with daunorubicin and vincristine and intrathecal chemotherapy with
methotrexate, cytarabine and desamethasone. Funduscopy and OCT, after one course of systemic chemotherapy and two courses of intrathe-cal chemotherapy, showed complete regression of the retinal infiltration with full recovery of visual function (Figure 2). OCT is a non-invasive way to study the retina that uses reflection of light off the retinal layers to create a high resolution colour tomographic image of retinal structures with an axial resolution of 10 microns or less. In leukemic patients with a suspicion of posterior ocular segment involvement this technique can be considered as a new and non-invasive diagnostic procedure to see beneath the surface of the retina, permitting detection and follow up of leukemic infiltrates.

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TREATMENT RESULTS OF ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDREN ACCORDING TO ALL-BFM 95 PROTOCOL
M.I. Spasova, A.A. Stoyanova, I.N. Moumdjiev, K.G. Sapunarova
Medical University-Plovdiv, PLOVDIV, Bulgaria

Aims. To evaluate feasibility of treatment of acute lymphoblastic leukemia (ALL) according to the BFM 95 protocol at a single centre in Bulgaria; to assess the 10-years disease-free survival (DFS) in children from all the risk groups; to find factors with prognostic impact on survival from ALL. Methods. We studied a cohort of 104 children (59 boys and 45 girls) with ALL, treated at a single centre from January 1996 to January 2006. The mean age of the study group was 6.7 years (from 2 months to 18 years). The chemotherapy and treatment stratification were identical to the ALL-BFM 95 protocol. Patients were stratified into 3 risk groups, based on age, initial white blood cells’ count, immunophenotypic, cytogenetics and response to initial treatment: standard-risk (SR), intermediate-risk (IR) and high-risk (HR) groups. Response to initial treatment was assessed by the steroid response on day 8 and hematological remission on day 33 after beginning of chemotherapy. Survival curves were calculated according to the Kaplan-Meier method and statistical significance of differences between curves was determined by the log-rank Mantel-Cox test. Logistic regression was carried out for assessing factors with prognostic impact on survival. Results. The patients from the study group were stratified in SR: 31 (29.8%), IR: 52 (50%) and HR: 21 (20.2%) patients. CNS involvement was proven in 2 (1.9%) patients, mediastinal mass - in 14 (13.5%) patients, renal infiltration - in 13 (12.5%) patients. Poor steroid responders were 20 (19.2%) patients and remission was not achieved on day 33 in 6 (5.8%) patients. The 10-years DFS probability was 81.4±1.3% for the SR group, 72.5±0.7% for the IR group and 58.6±1.3%, for the HR group (p<0.001). Independent prognostic factors for DFS, when conducting risk-adapted chemotherapy, proved to be radiologically-proven mediastinal mass (p=0.008; RR: 5.7, 95% CI: 3.2-41.6) and timely remission induction (p=0.002; RR: 24.6, 95% CI: 2.1-283.9). The majority of relapses occurred within 3 years from diagnosis and most involved the bone marrow. Conclusions. The DFS of the studied group is compatible with the reported from the official BFM study group. Our results on the basis of risk-adapted treatment suggest lack of correlation between survival and the prognostic factors considered previously as significant.

1178
COMPARISON OF HLA CLASS I (A, B, C) AND CLASS II (DRB) POLYMORPHISMS IN IRANIAN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) AND CONTROL GROUP
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Backgrounds. HLA gene polymorphisms have been extensively studied in various immune-mediated as well as malignant diseases such as leukemia. Since the first study on mouse leukemia by Lilly et al in 1964, the role of MHC molecules as genetic factors affecting the susceptibility or protection against leukemia have been proposed. Aims. The aim of this study was to compare the HLA class I (A,B,C) and class II (DRB) alleles frequency between a group of 141 Iranian patients with Acute Lymphoblastic Leukemia (ALL) and two distinct control group of 100 and 180 healthy individuals for HLA class I and II analysis, respectively. Methods. From all of the patients and control subjects blood samples were collected after giving informed consents. HLA class I antigens were determined using serology method while DNA extraction and HLA-DRB typing were performed using PCR-SSP analysis. Results. Significant increased frequencies of HLA-A*00: 12.4% vs. 1% (p<0.002, OR=0.074, 95% confidence interval (CI): 0.009-0.58) and HLA-Cw*07: 34.8% vs. 20% (p=0.03, OR=2.15, 95% CI: 1.13-4.08) were noticed in patients with ALL when compared with control group. Patients showed significant lower frequency of some antigens including HLA-B*05 (p<0.0001), B*12 (p=0.005), B*14 (p=0.005), B*41 (p=0.005), B*61 (p<0.0001), B*65 (p=0.0005), B*52 (p=0.03) and Cw*05 (p<0.0001) than healthy control group. No significant differences were found between patients and control group when compared for HLA-DRB allele frequencies. Conclusion: This study suggested the role of some HLA antigens including HLA-A*00 and HLA-Cw*07 as predisposing factors in susceptibility to ALL. While through the antigens with lower frequencies in ALL patients, HLA-B*05, B*61 and Cw*05 showed a stronger and more significant differences. Future studies are needed to confirm these associations in larger samples and investigate the role of specific subtypes using molecular techniques.
ANOREXIA-CACHEXIA RELATED HORMONES AT DIAGNOSIS AND DURING CHEMOTHERAPY IN CHILDREN WITH ACUTE LYMPHOBlastic LEUKAEMIA

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Backgrounds. Anorexia and cachexia are common manifestations in children with acute lymphoblastic leukaemia (ALL) at diagnosis. Possible mediators of the anorexia-cachexia syndrome are hormones, cytokines, and adipokines from peripheral tissues, and neurotransmitters, neuropeptides, and other hormones in the hypothalamus. Peptide YY (PYY) and ghrelin are gastrointestinal tract-derived hormones involved in the short- and long-term regulation of food intake and energy balance. PYY, synthesized mainly by endocrine cells of the terminal ileum and colon, is released into the systemic circulation in response to a meal and participates in signalling the end of the meal at the hypothalamus. PYY exerts its pro-satiety actions possibly through an Y2 receptor-mediated mechanism. Ghrelin, secreted predominantly from X/A-like endocrine cells of the oxyntic glands of the stomach, is primarily secreted in the fasting state, with plasma concentrations falling within one hour of a meal. Its role in food intake and energy balance is opposite to that of PYY, as it exerts orexigenic effects through activation of the hypothalamic neuropeptide Y-Y1 (NPY-Y1) pathway. Aims. We evaluated the secretion of PYY and ghrelin at diagnosis and during chemotherapy in children with ALL. Methods. Ten patients aged 2-7 years were included in this perspective study. All patients were treated following the same protocol (HOPDA97). A physical examination was performed and blood chemistries were evaluated by standard techniques. Preplantual PYY and active ghrelin levels were determined by specific radioimmunoassays (Linco Research, Inc., USA). Measurements were performed at diagnosis prior to chemotherapy and at several time points prior to each next cycle of chemotherapy for up to 18 months (6-10 measurements per patient). Results. Baseline PYY levels were 212.4±39.4 pg/mL, increased significantly to 283.9±72.9 pg/mL after the induction and consolidation phase of chemotherapy, and returned progressively to pre-treatment levels at the 6th cycle of the maintenance phase. Baseline active ghrelin concentrations at diagnosis were low (52.6±8.6 pg/mL), fluctuated throughout the study period and stabilized at significantly higher levels (57.4±31.6 pg/mL) after the 8th cycle of maintenance chemotherapy. Conclusions. These data suggest that in children with ALL and anorexia-cachexia the levels of PYY decrease with time, as the leukemic burden is eliminated. In contrast, active ghrelin levels are relatively low at diagnosis, remain low during the early cycles of chemotherapy, but normalize with the elimination of the leukemic burden, paralleling the body weight gain trajectory.

References

SCREENING FOR EVI1 ECTOPTIC EXPRESSION IN T-ALL PATIENTS

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Backgrounds. Balanced chromosomal rearrangements involving chromosome band 3q26 due to translocations with various partner chromosomes are a recurrent finding in myeloid malignancies. These translocations either contribute to the ectopic expression of, or to the formation of fusion genes involving the EVI1 proto-oncogene. EVI1 transcriptional activation has been reported in up to 10% of acute myeloid leukemias (AML), and is an independent indicator of adverse prognosis. While EVI1 expression is a well documented oncogenic event in myeloid malignancies, EVI1 was presumed not to be involved in lymphoproliferative disorders. However, in an extensive molecular characterization of unselected 3q26 rearrangements, we recently reported the sporadic occurrence of balanced 3q26 aberrations in lymphoid neoplasms. In a t(3;9)(q26;p23) identified in a T-cell Non Hodgkin’s lymphoma, FISH confirmed a genomic EVI1 rearrangement. Since these observations suggested a possible involvement of EVI1 in T-cell malignancies, we initiated translational analyses designed to define the presence and frequency of ectopic EVI1 expression in T-ALL. Aim of the study. The aim of this study was to investigate the possible ectopic EVI1 expression in T-ALL. Methods. We performed real-time quantitative PCR using validated EVI1 primer pairs (1), dedicated to the sensitive detection of ectopic EVI1 expression, on a multi-centre collected series of 87 T-ALL patients and 3 T-ALL cell lines. Results. EVI1 overexpression was demonstrated to be absent in the 87 patient samples and the 5 tested T-ALL cell lines. Conclusion: Although EVI1 overexpression is a poor prognostic marker in AML, it seems not to be involved in the pathogenesis of T-ALL.

References

ECONOMIC AND QUALITY OF LIFE BURDEN OF HIGH-RISK ACUTE LYMPHOBLASTIC LEUKEMIA

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Backgrounds. Patients with high-risk acute lymphoblastic leukemia (ALL), including Philadelphia chromosome positive (Ph+) ALL, typically have extremely poor prognosis, experience poor quality of life (QoL) and incur high economic cost. Aims. This study examined the economic and QoL outcomes for high-risk ALL patients including Ph+ ALL. Methods. A systematic search of the English-language literature published between 1990 and 2005 was conducted. Additional searches were conducted from the retrieved article bibliographies and appropriate conference proceedings (2000-2005). Articles selected for inclusion were prospective or retrospective studies specifically designed to examine burden of illness, direct medical costs, cost drivers, or QoL outcomes of ALL and treatments. Results. Of 798 abstracts screened, 106 met selection criteria and were reviewed in detail. Forty-nine and 47 studies focused on the economic and QoL aspects of ALL, respectively. Patients with high-risk ALL are usually defined by cytogenetic alterations (e.g. t(9;22)(q34;q11), t(4;11)(q21;q23)), age, increased white blood cell count, and slow response to therapy. The average annual direct medical cost per high-risk ALL patient ranged from $100,000 to $150,000 as compared to $40,000 to $74,000 for a standard-risk ALL patient. Hospitalization was the major cost component comprising 50%-80% of total direct costs. Major hospital cost drivers included infections, chemotherapy, growth factors, transfusions, and transplantation. These drivers resulted in more frequent hospitalizations and longer ICU lengths of stay for high-risk patients. High-risk ALL patients typically had psychological problems and physical complaints, especially in domains of emotion, cognition, and pain. Furthermore, high-risk patients were more likely to have poorer QoL than standard-risk patients due to higher relapse rates and increased need for transplantation. Conclusions. ALL exacts a substantial economic and QoL burden on patients, their loved ones and society in general. This burden appears particularly heavy for high-risk patients, such as patients with Ph+ ALL, one of the worst prognosis in ALL. Imatinib either as a single agent or as part of combination regimens has been reported to extend disease-free-survival and improved quality of life among patients with Ph+ ALL in clinical studies (Pui et al. NEJM 2006). Further research is undertaken to evaluate the economic and QoL benefits of imatinib as compared to the current therapies in the treatment of Ph+ ALL.

TREATMENT OF RELAPSED PHILADELPHIA-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA AFTER MYELOABLATIVE STEM CELL TRANSPLANTATION WITH IMATINIB

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Backgrounds. Philadelphia chromosome-positive acute lymphoblastic leukemia have a markedly poor prognosis when treated with conventional chemotherapy alone. Even with intensive treatment such as allogeneic transplant, a large proportion of patients relapse. Aims and Methods. We described here two patients with Ph+ ALL who relapsed after HLA-identical sibling donor stem cell transplantation and were treated...
with shortened course of reinduction chemotherapy and imatinib mesylate (400 mg/day). **Results.** One of these (male age 36) received imatinib for MRD positivity detected in PCR after SCT. Bcr-abl transcript became undetectable after 1.5 month of imatinib treatment. STI and immunosuppressive therapy was discontinued at day +120. These response was not sustained and the patient relapsed 9 month after alloSCT despite of chemotherapy. He was treated with imatinib combined with additional mild chemotherapy and achieved complete donor chimerism with PCR negativity. Because of extensive chronic GVHD (skin, liver, oral sicca, sclera, bronchiolitis obliterans without thrombocytopenia) systemic steroids therapy was introduced and imatinib (400mg/day) was simultaneously continued. At present, the patient has a 19-month post-transplantation follow-up and is in stable molecular remission as evaluated by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) for the BCR/ABL fusion gene transcript. GVHD has partially resolved and patient was able to reduce immunosuppression. In the notably during imatinib treatment we have observed unusual clinical improvement of bronchiolitis obliterans (BO) process confirmed by computed tomography and pulmonary function testing. Another patient relapsed 6 month after alloSCT and obtained complete hematological remission with 100% donor chimerism and PCR negativity after mild chemotherapy followed by imatinib. Imatinib was well tolerated and did not induce GVHD. At 18 months follow-up patient is still in complete hematological and molecular remission. DLI is planned. **Conclusions.** Imatinib combined with low dose chemotherapy is a promising therapy option in Ph+ALL patients relapsed after alloSCT not eligible for intensive treatment, achieving remission prior to DLI and maintain remission during immunosuppressive therapy. Further studies are needed to elucidate the role of imatinib in transplant BO are needed.

**1184**

**BURKITT-LIKE LYMPHOMA: A NEW TREATMENT PROTOCOL BLL-M-04**

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**Backgrounds.** Burkitt-like lymphoma (BLL) is a clinical and morphological variant of Burkitt lymphoma. It is the most aggressive B-cell lymphoid neoplasm, which proliferative activity approximates 100%. At the same time BLL is one of the most chemosensitive lymphoma. Intensive chemotherapy allows achieving remissions in 70-90% cases and increasing a common 5-year survival up to 60-75% (according to the stage of disease). **Aim:** to evaluate an efficacy and toxicity of protocol BLL-M-04. **Methods.** Seventeen patients (12 males and 5 females, mean age 25 years) were eligible for inclusion in the study if they had a diagnosis of Burkitt-like lymphoma (BLL). All the patients participated in the study performed in Russian Hematological Research Center between January 2006 and July 2005. Fourteen patients were eligible for recursive treatment previously. Three patients from another clinics had a diagnosis of Diffuse B-large cell lymphoma and were treated with CHOP (cyclophosphamide, vincristine, adriamycin and prednisolone), but in Russian Hematological Research Center were used additional diagnostic methods (fluorescent in situ hybridisation, flow cytometry, immunohistochemistry) and BLL was diagnosed. The BLL staging criteria developed by S. B. Murphy was used to stage the patients. The stage I, II, III, IV was diagnosed in 2, 1, 6, 8 patients respectively. Bone marrow was involved in 5 patients, neutropenia - in 5 patients. B-symptoms (night sweats, fever, weight loss) were in 13 patients. Serum lactate dehydrogenase level was increased in 15 patients. The remission rate of a new treatment protocol intensification and treatment duration reduction in patients with BLL. Our new treatment protocol basis on standard NHL-BFM-90 protocol. We know that BLL is a chemosensitive tumor and regresses after 1-2 courses of chemotherapy. Despite the initial tumor mass we decided to treat BLL according to 4 courses (2 inducational and 2 consolidational). According to the fact that BLL is the most sensitive to high dose methotrexate and cytarabine we used this drugs in the induction phase to achieve the most cytoreductive effect. Courses A and C were used for remission achievement. Doxorubicin was added to course A, methotrexate - to course C. Consolidative courses were the same as inducational courses. So, we used A and C courses (without B), intensified with course B drugs (doxorubicine and methotrexate), interval between courses was 21 days. **Results.** Seventeen patients were treated with BLL-M-04 protocol, 16 patients (94%) achieved a CR after 1-2 courses (9 patients - after 1st course, 7 - after 2nd). Fifteen patients are in a first CR during 16,5 months (median 5-27 months). Two patients were died. The course of death was traumatic subdural hematoma in patient with chemotherapy induced thrombocytopenia up to 4×10^10/L in CR. The 2d patient dead because of severe fungal sepsis during remission induction. Treatment duration was 3,0-3,5 months. Myelotoxic agranulocytosis completed all courses. The most number of infectious and hemorrhagic complications of treatment were registred during the first course A, that can be explained with induction therapy of BLL. The most number of relapses are after 8-12 months of treatment and after 24 months we can think about full recovery. So, in the nearest future we can evaluate an efficacy of a new treatment protocol BLL-M-04. The usage of this protocol can achieve a rapid BLL regression and treatment duration reduction because of treatment intensification and acceptable toxicity.

**1185**

**BURKITT'S LYMPHOMA IN KOREA: CLINICAL MANIFESTATIONS AND EFFICACY OF MODIFIED CALGB 9251 REGIMEN (BNHL)**

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**Backgrounds.** Clinical manifestations of sporadic Burkitt's lymphoma of Asia including Korea has not been well informed. When Korean patients were treated with a new treatment protocol proposed by the Cancer and Leukemia Group B (CALGB) 9251, grade 4 mucositis was reported in every patient. Thus, we had modified CALGB 9251 protocol and named BNHL. **Aims.** This study was aimed to show the clinical manifestations and to evaluate efficacy and toxicity of BNHL in patients with Burkitt's lymphoma in a Korean single center. **Methods.** Between January 1998 and July 2005, 25 patients who were diagnosed as Burkitt's lymphoma at Asian Medical Center were included. Among 25 patients, 12 patients were treated with BNHL and 13 patients were treated with CALGB 9251 protocol and named BNHL. **Results.** Median age was 50.4 years (range: 17-77) and 15 patients were in stage I, 2 patients in stage II, 3 patients in stage III, and 5 patients in stage IV. Regarding initial presentation, 1 patient was with acute leukemia - in 5 patients. B-symptoms (night sweats, fever, weight loss) were in 18 patients (91%), and 13 patients (65%) had extranodal involvement. Serum lactate dehydrogenase level was increased in 22 patients (92%). Median overall survival (OS) was not reached yet, and 1-year OS rate was 64% (95% CI, 50-76%). All patients treated with BNHL had hematologic toxicities of grade 3 or 4 neutropenia/thrombocytopenia. Grade 3 or 4 non-hematologic toxicities were: mucositis (67%), infection (58%), hepatic toxicity (42%), peripheral neuropathy (25%), and azotemia (25%). **Summary/Conclusions.** Korean patients with Burkitt's lymphoma had worse age-adjusted IPI score than those in western countries, but in Korean BNHL protocol, we reduced the dose of etoposide (50-50 mg/m^2/day on days 4 and 5 of cycles 2, 4, and 6. **Results.** Median age was 50.4 years (range: 17-77) and 15 patients were male. Among 25 Burkitt's lymphoma, 20 patients had extranodal involvement, and 9 patients had 2 or more extranodal involvements. Extranodal sites were bone marrow, GI tract, genitourinary organ, bone, and lung, in decreasing order. Among total patients, 16 were in stage III or IV, and 9 were in stage I or II. Twelve patients had B symptoms, and 16 patients had high or high intermediate score of age adjusted international prognostic index (IPI). For 12 patients treated with BNHL, median follow-up duration was 13 months (range: 3-20 months). Among patients treated with BNHL, 9 patients achieved CR, and 3 patients achieved PR. The event free survival (EFS) rate at 1 year was 54% (95% CI, 39-69%) and median EFS was not reached. Among 9 patients who achieved CR, 2 patients were relapsed. Three patients died as a result of treatment-related complications (2 patients) or progressive disease (1 patient). Median OS rate was not reached yet, and 1-year OS rate was 64% (95% CI, 50-76%). All patients treated with BNHL had hematologic toxicities of grade 3 or 4 neutropenia/thrombocytopenia. Grade 3 or 4 non-hematologic toxicities were: mucositis (67%), infection (58%), hepatic toxicity (42%), peripheral neuropathy (25%), and azotemia (25%). **Summary/Conclusions.** Korean patients with Burkitt’s lymphoma had worse age-adjusted IPI score than those in western countries, but in Korean BNHL protocol, we reduced the dose of etoposide (50-50 mg/m^2/day on days 4 and 5 of cycles 2, 4, and 6.

**1186**

**ACUTE PERIPHERAL NEUROPATHY FOLLOWING HYPERCVAD REGIMEN FOR MANTEL-CYTE LYMPHOMA**

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**Backgrounds.** HyperCVAD is an effective regimen for the mantle cell lymphoma (MCL) with potential cerebellar toxicity, but non acute peripheral neuropathy like Guillain-Barre syndrome has been reported. We report three patients with MCL that present with acute polyneuropathy probably secondary to the hyperCVAD/MTX-AraC reg.
imem. Methods, retrospective study of MCL patients treated in our Hospital with the hyperCVAD regimen and review of the literature. Results: Case 1: 54 years old male with a history of polyarteritis nodosa and cerebral infarction, diagnosed in 1996 of MCL. Four weeks after the second course (MTX-AraC) the patient presented with proximal pain and weakness in arms, than in hours progressed to the legs, and in two days to a quadriplegia and inability to swallow. An electromyography (EMG) showed a motor demyelinating polyneuropathy. An MRI and CSF study didn’t showed neurological infiltration. The patient was treated with e.v. immunoglobulin with a partial recovery of strength. Six months later the lymphoma relapsed and the patient died. Case 2. A 58 years old male with a history of chronic renal failure, an incipient polyneuropathy and two cadaveric renal transplantations, that was diagnosed of MCL in 1998. Three weeks after the four hyperCVAD courses (the MTX-AraC was omitted) the patient presented a progressive and severe weakness in legs and pain in proximal muscles of limbs, and four days later the patients need to bed resting. An EMG showed a motor and sensorial axonal polyneuropathy. An MRI and CSF study didn’t showed neurological infiltration, and the lymphoma was in complete response. There was non response to e.v. immunoglobulin therapy and the patient died by a sepsis. Case 3. A 53 years old man with a history of diabetes mellitus diagnosed in 2005 of MCL and treated with Rituximab-hyperCVAD/MTX-AraC. Three weeks after 5 course (3 hyperCVAD and 2 MTX-AraC) the patient started with peroneal paresis and proximal torsion of dorsal muscles. A week later the patient presented with posis, a more severe muscular pain, a depressed tendon reflexes and a progressive weakness in the extremities, more severe in the upper extremities. An EMG showed a motor and sensorial axonal polyneuropathy. An MRI and CSF study didn’t showed neurological infiltration. The patient was treated with e.v. immunoglobulin, metilprednisolone and plasmapheresis with only a temporal improvement. After one month the pain ceased but the neuropathy progressed with a complete quadriplegia, bladder atony, and a deficient vibration and positional sensory. Four months later the lymphoma relapsed and the patient died. Conclusion. The hyperCVAD is an intensive regimen with significant toxicities and we think that acute peripheral neuropathy was a toxic manifestation. A previous peripheral neuropathy or risk factor as uremia and diabetes mellitus could predispose our patients to developing a severe polyneuropathy. These features must to take into account before the choice of hyperCVAD regimen.

1188 LIPOSOMAL DOXORUBICIN IN THE TREATMENT OF LYMPHOMA PATIENTS
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Backgrounds. Myocet (liposomal doxorubicin) has an improved pharmacokinetic profile with less myelosuppression and GI toxicity and has a reduced risk of cardiotoxicity at dose level equivalent to standard formulations of doxorubicin. Methods. From June 2003 we replaced the conventional doxorubicin with liposomal doxorubicin (Myocet 50 mg/m² in COMP and 25 mg/m² in MBVD) for the treatment of 25 patients (pts). 12 pts were selected pts: elderly pts, pts with impaired cardiac function, pts previously treated with less myelosuppression and GI toxicity. Twelve pts with NHL were treated with R-COMP and 5 Hodgkin’s lymphoma with MBVD. Results. The median age was 68 years (range 54-76). Three pts were stage I, 7 stage II, 6 stage III and 9 stage IV. According to IPI score, for NHL only, 7 pts were low risk, 6 low-intermediate, 6 intermediate-high and 1 high risk. Seven were pretreated with doxorubicin (490 mg median cumulative dose), 7 pts showed impaired cardiac function (4 ischemic, 7 hypertensive and 2 hypokinetic). The median left ventricular ejection fraction (LVEF) at diagnosis was 59% (range 45%-70%). All patients performed cardiac evaluation at diagnosis, after three cycles and at the end of therapy. All pts but one had no change in LVEF, one patient (4%) presented a myocardial dysfunction resolved with medical therapy. The average dose of liposomal doxorubicin for patients who concluded therapy was 465 mg (range 80-600 mg). At the moment 21 out 25 patients are evaluable for response: 15 pts obtained a complete response (60%), 2 partial remission (8%), 2 stable disease (8%), 4 progressive disease (16%). According to IPI score, for NHL only, 7 pts were high risk, 11 low risk, 7 low-intermediate. Comparing the IPI score, the response was statistically significantly (p < 0.05) better in patients with a low IPI score. The median number of cycles administered was 13 (range 1-21) and the median number of days exposed to treatment was 75 (range 4-455) days. The overall survival was 80%. Conclusions. We conclude that liposomal doxorubicin allows to treat patients with concomitant diseases which could limit the use of conventional anthracyclines. Myocet is feasible and effective in a subset of patients with very negative characteristics at diagnosis. It reduces cardiotoxicity risk without reducing chemotherapeutic efficacy.

1187 PHASE II CLINICAL EXPERIENCE WITH BORTEZOMIB IN PATIENTS WITH INDOLENT NON-HODGKIN’S LYMPHOMA AND MANTLE CELL LYMPHOMA
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Backgrounds. Bortezomib is a novel small molecule, which is a potent selective and reversible inhibitor of the proteasome. Bortezomib has also shown activity in vitro against a variety of lymphoma cell lines including mantle-cell lymphoma (MCL)-derived and diffuse large-cell lymphoma-derived cell lines. Aims. The preclinical results and clinical observations provided the rationale for this Phase II trial of Bortezomib in patients with relapsed or refractory B-cell non Hodgkin Lymphoma. Methods. To date, we have treated 11 previously treated patients (pts.), (8 males and 3 females with a median age of 60 years, median number of prior therapies 4) with relapsed or refractory indolent lymphomas including: 2 pts. with small lymphocytic lymphoma; 2 pts. with follicular lymphoma; 7 pts. with MCL. Patients were treated at a dose of 1.5 mg/m² twice weekly for two consecutive weeks with a one-week rest period. Results. No grade III or IV toxicities were observed, save one patient that developed a grade 3 sensory and motor neuropathy. Re-staging studies were routinely performed after two complete cycles of therapy. All pts. with small lymphocytic lymphoma and follicular experienced PD. Among the seven assessable pts. with MCL there was two pts. with CR, two pts. with PR, one patient with SD in two pts. and PD. In responders pts., the median time to progression was not reached with a median follow-up of 9.1 months. Conclusions. These data continue to support the biological activity of Bortezomib in pts. with select sub-types of indolent non-Hodgkin’s Lymphoma.

1189 INTERIM ANALYSIS OF MULTICENTER PHASE II TRIAL OF GEMCITABINE, ETOPOSIDE, CISPLATIN AND DEXAMETHASONE (GEPD) CHEMOTHERAPY IN PATIENTS WITH PRIMARY REFRACTORY OR RELAPSED NON-HODGKIN’S LYMPHOMA (NHL)
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Backgrounds. Platinum and etoposide-based chemotherapy has been used extensively as salvage therapy for NHL. Gemicitabine was studied in a number of phase II trials as single agent against relapsed NHL. In these studies, the single-agent gemcitabine as salvage treatment showed moderate activity and mild toxicities against the heavily pretreated lymphoma patients. Aims. We access the efficacy and safety of GEPD chemotherapy (gemcitabine 700 mg/m² continuous i.v. on day 1, 80 mg/m² i.v. on day 1-4; etoposide 40 mg/m² i.v. on days 1-4; cisplatin 60 mg/m² i.v. on day 1; dexamethasone 40 mg i.v. on days 1-4) in relapsed or refractory NHL patients. Courses were repeated every 21 days. Methods. Patients with histologically proven diagnosed NHL, documented relapse or resistant disease were eligible. All patients received GEPD chemotherapy as salvage treatment. The primary end point was a response rate after 2 cycles. Patients could then proceed to stem cell collection using mobilizing regimens (ESAP or GEPD plus filgrastim) and followed by autologous stem cell transplantation or continued to additional 4 cycles of GEPD. Results. Between Jan 2005 and Dec 2005, 15 patients (8 males and 7 females) were enrolled in the study. Median age was 55 (range 16-75) years. Of these patients, 7 patients (46.7%) had diffuse large-B cell lymphomas. Median follow-up duration was 5.1 (range 1.0-13.0) months. All patients received two cycles of GEPD chemotherapy: 2 partial response. There was an overall response rate of 46.7%. Myelosuppression was the dose-limiting toxicity. 11 patients (73.3%) experi-
enced grade 4 neutropenia and 6 patients (40.0%) experienced grade 4 thrombocytopenia. Autologous stem cell collection was attempted in the 7 patients and was successful in all cases. The median number of CD34-positive cells collected was 5.2 (range, 2.8-11.6)×10^6/kg. Of 13 patients <66 years, 4 patients (30.8%) proceeded to stem cell transplantation. Conclusions. GEPD chemotherapy in patients with primary progressive or relapsed NHL is effective as salvage therapy and does not interfere with the ability to harvest autologous stem cells for subsequent transplantation. A final analysis is planned after total 40 patients are enrolled.

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R-FMD vs R-CHOP TREATMENT AS FIRST LINE THERAPY FOR FOLLICULAR LYMPHOMAS: A SINGLE INSTITUTIONAL EXPERIENCE
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Background. High response rates in follicular lymphoma (FL) with the FMD protocol have been previously reported. The monoclonal anti-CD20 antibody Rituximab has been shown to induce a high response rate in FL patients and to improve outcome when associated with classic regimens (CVP or CHOP). Aims. We have evaluated the impact of R-FMD as compared to R-CHOP as a first line therapy in patients with follicular lymphomas, in terms of: complete response (CR), overall survival (OS), toxicity and the efficacy of PCR molecular analysis in predicting clinical and molecular remission. Methods. Between September 2002 and April 2005, 155 FL patients were enrolled in the study. Patients were assigned to 2 arms: (M/F: 8/6, median age 54 years) received R-FMD treatment in stage II-IV, FL-IPI score: intermediate grade 3 pts, high grade 11 pts. R-FMD regimen was administered every 28 days for six cycles: Fludarabine 30 mg/m^2 e.v. days 1-3, Mitoxantrone 10 mg/m^2 day 1, Dexamethasone 20 mg days 1-3 and Rituximab 375 mg/m^2 day 1. PCR molecular analysis was performed in 12 patients at diagnosis, showing in 10 (84%) of them bcl-2 rearrangement. Seventeen patients (M/F: 7/7, median age 56 years) received R-CHOP treatment in stage II-IV, FL-IPI score: intermediate grade 4 pts, high grade 10 pts. R-CHOP regimen was delivered every 21 days for six cycles, preceded on day 1 by Rituximab 575 mg/m^2. PCR molecular analysis was performed in 10 patients at diagnosis showing in 9 (90%) of them bcl-2 rearrangement. Results. Arm R-FMD: An overall response (13 (93%) CR and 1 (7%) partial response) was achieved in all patients; the pts in PR achieved CR after Zevalin. Actually, after a median follow up of 28 months, all 14 pts resulted in CR. At the end of treatment, bcl-2 appeared to be negative in 8/10 pts (75%). The toxicity was mild with grade 3-4 neutropenia in 2 pts (14%). CMV infection was observed in one pt. Arm R-CHOP: Thirteen pts (95%) achieved CR and 1 resulted non responder. Out of all 13 pts in RC: 1 died in CR for infection, 1 relapsed after 23 months. After a median follow up of 25 months, 12 (86%) pts are alive, 11 (78%) of which are in continue CR. Grade 3-4 neutropenia was observed in 4 (28%) pts. At the end of treatment bcl-2 appears to be negative in 6/9 pts (66%). Conclusion. Our data demonstrate that both frontline R-FMD and R-CHOP treatments produce high rate of response in terms of CR, OS and molecular remission and low toxicity. A more prolonged follow-up will be needed to determine the long-term efficacy of these combinations.

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T(14;18), PsS AND RAS GENES MUTATION IN PATIENTS WITH RESIDUAL LYMPHOMA CELLS
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PCR analysis of rearranged antigen receptor genes reaches sensitivity of 10^-6 and has been demonstrated as valuable tool for detection of minimal residual disease (MRD) in lymphoma malignancies. However, the finding that patients with evidence of MRD sometimes remain in long-lasting remission directs further investigations toward biology of residual disease. The aim was to correlate MRD results with the incidence of relapse and DF, respectively. Furthermore, the presence of PS3 and RAS gene mutations and t(14;18) was analysed in patients with residual malignant cells. The study included 40 B-NHL patients diagnosed and managed in MMA. 13/40 patients had high- (HG) and 27/40 had low-grade (LG) lymphoma. Seven patients achieved partial (PR) and 33 patients achieved complete clinical remission (CCR) after chemotherapy. Peripheral blood samples were analysed for MRD at up to ten follow-up points. All analysis included PCR amplification followed by appropriate electrophoresis. MRD was found in 13/33 patients (12 LG and 1 HG) who achieved CCR. The incidence of relapse was significantly higher in MRD+ vs MRD- NHL patients (Fisher’s exact test, p=0.0053). In the LG group significant difference was not found. The only MRD+ HG patient relapsed. Significant difference in DF between MRD+ and MRD- NHL patients was not observed. Concerning MRD+ patients in CCR and patients who achieved PR, t(14;18) was found in six patients (4 relapsed). In the same group PS3, K- and N-RAS mutations were not found. H-RAS mutations were found in six patients - 3 relapsed and 3 remains in CCR. Our results demonstrated positive correlation between MRD + positivity and incidence of relapse in B-NHL patients, relapse did not indicate significance of PS3 and RAS mutations for evaluation of residual clone malignancy.

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DOSE-ADJUSTED EPOCH-RITUXIMAB IS HIGHLY EFFECTIVE AND TOLERABLE IN UNTREATED POOR-PROGNOSIS DIFFUSE LARGE B-CELL LYMPHOMA: RESULTS OF A PROSPECTIVE STUDY
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Less than 50% of patients with poor-prognosis diffuse large B-cell lymphoma (DLBCL), defined as score 2 and 3 (intermediate-high or high) according to the age-adjusted International Prognostic Index (aaIPI), remain disease-free for lengthy periods. The optimal therapeutic strategy for these patients is still much debated. This study was to evaluate the EPOCH (DA-EPOCH-R) regimen for untreated poor-prognosis DLBCL. DA-EPOCH-R regimen (doxorubicin, vincristine, etoposide over 96 hours’ infusion with bolus cyclophosphamide, prednisone, and rituximab) was administered to 31 consecutive patients with previously untreated poor-prognosis DLBCL. At the end of DA-EPOCH-R, consolidation rituximab radiotherapy (26 Gy) was given to areas of previous bulky disease (≥10 cm). The last 8 patients received an intensive central nervous system (CNS) prophylaxis consisting of 3 courses (after cycles 2, 4 and 6 of DA-EPOCH-R) of oral dexamethasone (40 mg on days 1-4) and intravenous high-dose methotrexate 3 g/m^2 (1.5 g/m^2 in patients > 60 years of age). 12 Gy infusion, day 1 (with folic acid rescue). Median cycles of EPOCH regimen administered were 6 (ranged from 2 to 8 cycles). Younger patients (aged < 60 years) required higher dose rates than older patients (>60 years) to achieve the targeted absolute neutrophil count (ANC) nadir. Two-hundred and six cycles of chemotherapy were administered to 51 patients. Of the 51 patients enrolled in the study, 28 were evaluable for response. Overall, 92% of patients had an objective response; 76% (22/28) achieved a complete response (CR) and 14% (4/28) had a partial response (PR) at the end of treatment. At a median follow-up of 23 months (range 9-45), the event-free survival (EFS) and overall survival (OS) were 71% and 85% respectively. Two CR patients (both with an aaIPI score of 3) relapsed. Only aaIPI score of 3 demonstrated to have an adverse prognostic value. Toxicity at least grade 3 according to the WHO toxicity criteria (incidence by cycle) were: neutropenia (55%), thrombocytopenia (25%), anemia (15%), and oral mucositis (6%). Severe infections occurred in 25% of the cycles in patients older than 60 years of age compared with 10% of cycles in patients younger than 60 years of age. Four patients with previous cardiac disease and 3 patients with HCV antibody showed no severe cardiac nor hepatic toxicity during chemotherapy. There were 2 toxicity-related deaths (treatment-related mortality, 6%): one patient had an early toxic death due to neutropenic sepsis at week 16, and the other patient had a late toxic death due to secondary acute myeloid leukemia (FAB M4) that occurred at 8 months after the end of DA-EPOCH-R. The data from our institution are promising and add to the available evidence supporting the efficacy and safety of DA-EPOCH-R therapy for the treatment of poor-prognosis DLBCL, especially in patients with an aaIPI score of 2.

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CHOP VS. RITUXIMAB-CHOP IN DIFFUSE LARGE B-CELL LYMPHOMA: A RETROSPECTIVE COMPARISON OF RESPONSE RATES AND OUTCOME
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Backgrounds. Rituximab is an anti-CD20 monoclonal antibody that
induces cytotoxicity via antibody-dependent cell-mediated and complement-dependent mechanisms, as well as via direct apoptotic signal- ing. Combination of rituximab with chemotherapy has an additive or synergistic effect and has been reported to increase response rates and prolong remission and survival in patients with diffuse large B-cell lymphoma (DLBCL). Aim: To evaluate and compare retrospectively the response rates and outcome of a large number of patients with DLBCL according to the kind of treatment administered, CHOP-like regimens vs. rituximab-CHOP. Methods: Between 1997 and 2004, 204 consecutive patients were diagnosed with DLBCL in our department. Patients were divided in two groups according to the kind of treatment administered. Group A comprised 113 (55.4%) patients, that received CHOP and CHOP-like regimens; 81 (71.5%) patients died from recurrent lymphoma. Group B consisted of 91 (44.6%) patients, that additionally received rituximab 375 mg/m² IV on day 1 of each chemotherapy cycle. Patients in both groups underwent a median number of 6 (1-8) cycles. Radiotherapy was additionally administered in 24 (21.2%) patients of group A and in 28 (30.8%) patients of group B (p < 0.05). Patients' characteristics (gender, age, nodal or extranodal primary site of origin, stage, IPI, presence of B symptoms, extranodal involvement other than primary, bulky disease and bone marrow infiltration), as well as response rates, were compared between the two groups using χ² tests. Disease-free survival (DFS), overall survival (OS) and failure-free survival (FFS) were estimated according to the Kaplan-Meier method. Differences in survival rates were assessed using the log-rank test. Results: Patients were well-balanced regarding their characteristics (p > 0.05). Median follow-up time for groups A and B was 62 (1-99) and 29 (1-62) months respectively (p = 0.001). On an intention-to-treat basis, complete response rates were similar between groups A and B (85.5% vs. 89% respectively, p = 0.05). Actuarial 5-year DFS rate was significantly higher in group B vs. group A (94.4% vs. 72.6% respectively, p = 0.046). Actuarial 5-year OS and FFS rates were not significantly different between groups A and B (77.7% vs. 70% and 62.5% vs. 69.7% respectively, p = 0.05). Conclusion: According to our results, the addition of rituximab to chemotherapy yields a higher DFS rate than chemotherapy alone, in patients with DLBCL. Nevertheless, our study failed to confirm the superiority of the rituximab-chemotherapy combination in terms of OS and FFS rates, probably due to the significantly shorter follow-up of this group of patients.

### 1195
**CNS LYMPHOMA AND THE USE OF INTRACRANIAL RITUXIMAB: REPORT OF THREE CASES**

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**Backgrounds.** Central nervous system (CNS) involvement is an adverse prognostic factor for patients with non-Hodgkin’s lymphoma (NHL). Because of the limited passage of rituximab through the blood-brain barrier, intrathecal administration of rituximab has been considered as a possible treatment for CNS lymphoma. Methods. 5 patients with recurrent or persistent CD20+ primary parenchymal CNS NHL were treated with rituximab and chemotherapy. Results. Treatment with rituximab yields a DFS and OS rate of 75% and 87% at 3 years respectively. Conclusion. Rituximab is an efficient treatment for CNS lymphoma, with a manageable toxicity profile.

### 1196
**GOOD RESPONSES OF PRIMARY MEDIASTINAL B CELL LYMPHOMA (PMBCL) AFTER CHEMIOIMMUNOTHERAPY (CHOP-14-rituximab) CONSOLIDATED BY BEAM AUTO SCT AND IRRT**


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PMBL is a distinct entity in WHO classification prior described as DLBCL variant. It presents as mediastinal bulky tumor, locally invasive to adjacent mediastinal structures. The bone marrow is involved only in 2% of cases. Relapses tend to be extranodal, including central nervous system, liver, kidneys. Prognosis in PMBL treated by CHOP regimen is poor in most cases resistance of lymphoma cells occurs already during the first line chemotherapy and 5 year survival is about 20%. Patients with PMBL were 2005-2005 12 PMBC-L patients were treated at Hematology Department in Krakow. The median age patient group was 37.2. In 9 cases the disease was limited to mediastinum (stage I) according Ann Arbor, and subsequent 8 patients had more advanced disease with a spread to vertebral column, lungs or adjacent muscles. B symptoms were present in all cases. None of the patient had bone marrow involvement. The majority of patients had elevated LDH (medium 901/uL) and bulky disease at diagnosis (mediastinal mass >20cm) was present in 7 patients, more than 30 cm in 3 patients. IPI was a poor outcome predictor, as it was low (0-1) in 10 cases and intermediate (2-3) in 2 cases. Treatment schedule and results. Patients with PMBL were treated either with intensive chemotherapy/CHOP-14-Rituximab according GLSG (10 patients) or ACVBP chemotherapy according GELA (2 patients). In 8 patients - a good partial response to first line chemotherapy was consolidated by BEAM conditioned by auto SCT. All patients
received IFRT. Although further tumor regression after the transplant was moderate, so far 5-50 months after transplantation DFS is 87%. Four further patient were not transplanted due to denial, ineffective stem cells collection or poor performance status. 2 of them are in CR, and a 3rd one in a non progressive PR (residual mass 0). Residual masses observed in most of the patients (8/12) at the end of the first line therapy. In the whole group 2 year OS and EFS are 80 and 98% respectively. Conclusion. Intensive chemoimmunotherapy does change the prognosis of PMBCL patients although the role of transplant as the first line therapy remains debatable. Effect of radiotherapy is not proven, however similarities between PMBCL and Hodgkin Disease (gene expression analysis, common residual masses at the end of therapy, usually localized disease) make it a tempting therapeutic option.

### 1197

NATURAL KILLER/T-CELL LYMPHOMA: A SERIES OF SIX CASES FROM A SINGLE WESTERN INSTITUTION


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**Backgrounds.** NK/T-cell lymphoma is a rare entity mostly being reported in Asian countries; representing 6% of total of lymphomas. In western countries its incidence is lower and not well described. Neoplastic cells show an angiocentric pattern of infiltration and usually express CD2, CD3ε, CD56, TIA and granzyme B. **Aim.** We described a series of six Caucasian patients diagnosed of NK/T “cell lymphoma at our institution in Spain in the last 10 years, representing 0.8% of all patients.

**Patients and Methods.** Median age was 53 years (from 36 to 75), male sex 5/6 (83%). Five patients presented with involvement of nasal and paranasal cavities and one has a non-nasal type. Patients with nasal involvement presented with nasal obstruction and bleeding. B symptoms were present in 2/6 (33%) patients, High LDH 3/6 (50%), 5 of the five patients tested had a positive Epstein-Barr serology. Clinical Ann Arbor staging was: I 3 (50%), II 1 (17%) III 1 (17%) IV 1 (17%) and a modified staging system for paranasal lymphomas was T1 2 (33%), T3 1 (17%), T4 2 (33%). International prognostic index was: low risk 4 (66%), low-intermediate 2 (33%). A modified international index was score 0: one patient, 1 two, 2 two and 3 one patient. 3 out of six patients received chemotherapy including anthracyclines (mostly CHOP) as front line therapy and 2 received radiotherapy and one chemoradiotherapy as a rescue for progression following front-line chemotherapy. Results. Three of the five evaluable patients achieved a complete remission to front line chemotherapy, one achieved a partial response and one of them progressed immediately after chemotherapy (1) and radiotherapy (1). One patient progressed four months after finishing treatment. 2 of the six complete responders relapsed at three and six years after achieving response, all of them with disseminated disease and died as a consequence of lymphoma. Only 2 of the six patients remain alive at the moment. **Conclusion.** NK/T lymphoma patients diagnosed at our institution presented with clinical features and an aggressive outcome comparable to those described in eastern countries.

### 1198

HIGH-DOSE CHEMOTHERAPY WITH TANDEM AUTOLOGOUS TRANSPLANTATION IN RELAPSED/REFRACTORY HODGKIN’S DISEASE - A SINGLE CENTER EXPERIENCE

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**Backgrounds.** High-dose chemotherapy with autologous stem cell transplantation (auto-HSCT) is commonly used in relapsed/refractory cases of Hodgkin’s disease (HD). **Aim.** We report the results of tandem auto-HSCT in patients with relapsed/refractory HD. **Methods.** Thirteen patients were included in this study. The median age was 26 years (range 20-39). Disease status at first auto-HSCT was refractory relapse (n=4) or primary refractory (n=9). Before tandem transplantation all patients received ≥ 2 lines of chemotherapy, one patient received radiotherapy, two relapsed after previous auto HSCT. In eleven patients, only peripheral blood cells were used and in two patients both bone marrow and peripheral stem cells were used. Conditioning regimen with dexamethasone, BCNU, etoposide, cytarbamine, melphalan ( DexaBEAM) was used for the first transplant and busulfan and cyclophosphamid (BuCy2) in ten patients, and treosulan and cyclophosphamid in three patients for the second transplant. **Results.** Hematological reconstitution was complete in all patients at both transplants. The median time to neutrophil recovery (absolute neutrophil count ≥5/G/l) after first and second transplant was respectively: 11 days (range 7-16) and 12 days (range 9-16) and platelet recovery (platelet count ≥20/G/l) was respectively: 14 days (range 10-19) and 21 days (range 12-60). After first transplant only neutropenic fever and confirmed bacterial/fungal infections were observed, treated with granulocyte colony stimulating factor. After second transplant only transient congestive heart failure with ventricular arrhythmia and veno occlusive disease was recognized in two patients. One patient (8%) died due to treatment-related toxicity (veno-occlusive disease). In the treosulan group no serious complications was observed. With the median follow-up of 42 months (range 12-71) ten patients are alive (77%), eight are in remission (61%), four patients relapsed (23%). **Conclusion.** Dose-intensive chemotherapy with tandem transplantation is an option in selected patients with resistant/refractory HD who have poor prognosis and limited treatment opportunity.

### 1199

IFOSFAMIDE PLUS VINORELBINE SALVAGE THERAPY FOR REFRACTORY OR RELAPSED HODGKIN LYMPHOMA: 23 CONSECUTIVE CASES FROM A SINGLE TEAM

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**Aims.** To evaluate the efficacy and toxicity of ifosfamide and vinorelbine therapy in a heavily pretreated patient population before or after autologous stem cell transplantation (ASCT). **Patients.** Twenty-three patients were treated between 2000 Nov and 2006 Feb. The median age at the time of treatment was 28 (18-44) years. The combination was used as first salvage therapy in 11 patients following ASCT for resistant disease or relapse, whereas 12 patients received this treatment prior to or without ASCT. All of these 12 patients were treated with ifosfamide and vinorelbine as third or fourth-line therapy. Six of them had primary progressive disease, 3 had early relapse and 3 had late relapse after standard dose therapy. **Methods.** Ifosfamide (3 g/m²) days 1-4 by continuous infusion and vinorelbine (25 mg/m² i.v. days 1 and 4) were administered with G-CSF support and uromitexane uroprotection. The median number of the therapeutic cycles was 2 (range 1-9). **Results.** The response rate was 65%, with 11 complete (CR 48%) and 4 partial remissions (PR 17%). Of the 15 responding patients, 8 received ASCT, 4 underwent autologous stem cell transplantation with reduced intensity conditioning (NSCT), 2 received further chemotherapy because of progression and 1 had no more therapy and remained in long-term (56 months) complete remission. The regimen was successfully used to mobilize peripheral stem cells in 8 patients (the median number of collected CD34+ cells was 5,05×10⁶/kg), while 3 patients did not mobilize. The main toxic effect was grade IV neutropenia, documented in 96% of cases, however, hematologic toxicity was mild. **Conclusions.** The combination of ifosfamide and vinorelbine proved to be effective to minimize tumor burden before ASCT and NSCT with tolerable toxicity profile. One of our patients
who relapsed following ASCT and had no donor, has remained in continuous complete remission for 56 months.

1200 COMPARISON OF THE EXPRESSION OF DIFFERENTIATION MARKERS BETWEEN DIFFUSE LARGE B-CELL LYMPHOMA OF NODAL AND EXTRANODAL PRIMARY ORIGIN

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Backgrounds. Diffuse large B-cell lymphoma (DLBCL) is a biologically and clinically heterogeneous lymphoma. DLBCL can be divided into prognostically important subgroups with germinal B-cell (GC) and activated B-cell (non-GCB) types with a favorable and an unfavorable prognosis using a CDNA microarray. The expression pattern of differentiation markers CD10, BCL6 and MUM1 by immunohistochemistry (IHC) has been proposed as a surrogate to distinguish GC from non-GCB types. The purpose of our study was to compare the expression frequencies of a panel of B-cell differentiation markers and proliferation rate in DLBCL according to the primary site, lymph node, or different extranodal organs. Methods. This study included 50 cases de novo DLBCL, 25 of nodal origin and 25 of primary extranodal origin. Sites of extranodal disease were: stomach (8), spleen (6), testis (4), skin (2), ovary (1), pancreas (1), lung (1) and large bowel (1). All the tissue samples were formalin-fixed paraffin sections obtained by biopsy before chemotherapy. The tumors were subclassified according to WHO classification and evaluated by IHC. To define each case as GCN and non-GCB type a panel of 3 antigens, CD10, BCL6 and MUM1 was evaluated following the algorithm reported by Hans C. E. et al (Blood 2004;103;275). All samples were further analyzed for the expression of bcl-2. Immunoreactivity was judged to be positive if 20% or more tumor cells were stained. The proliferation rate was evaluated by percentage of Ki-67 positive tumor cells. Results. All tumors were CD20 positive. In nodal DLBCL CD10, bcl-6 and MUM-1 were positive in 17/25 (68%), 15/25 (60%) and 16/25 (64%) cases. Nine cases (36%) were classified into GC type and 16 (64%) into non-GCB type. Ten cases (40%) were bcl-2 positive, acti-

1201 T-CELLS DO NOT SUPPORT OSTEOCLASTOGENESIS IN AN IN VITRO MODEL DERIVED FROM NON-HODGKIN LYMPHOMA WITH OSTEOLYTIC LESIONS

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Bone involvement from hematological malignancies other than multiple myeloma (MM) and adult T cell leukemia (ATL) is an uncommon event. It is characterized by osteolytic lesions, whose underlying molecular mechanisms remain ill defined. With regard to non-Hodgkin lymphoma (NHL), osteolytic lesions have been reported to occur in about 5-15% of all cases, rarely as a presenting manifestation of disease. By contrast, MM associated lytic bone disease, observed in 70-80% of MM patients, is well recognized. It appears to be regulated by a complex signalling system, that involves the receptor activator of nuclear factor (NF)-κB (RANK), the receptor activator of nuclear factor (NF)-κB ligand (RANKL) and osteoprotegerin (OPG). In particular, RANKL is a potent osteoclastogenic factor, which also modulates immune response, lymphoma proliferation, lymphoma progression and osteolytic formation in vitro. RANKL is expressed on malignant cells, osteoclasts, bone marrow stromal cells, CD4+ CD8+ lymphocytes, and activated T cells. OPG competes with RANK for binding to RANKL preventing its osteoclastogenic effect, and can act as a decoy receptor for TNF-related apoptosis inducing ligand (TRAIL), exerting an antiapoptotic effect. A linkage between immunoregulation by T cells and bone loss has been found in MM and other bone loss associated diseases, as we demonstrat-

1202 THE PREVALENCE OF ANTI HCV ANTIBODIES IN B-CELL NON-HODGKIN'S LYMPHOMA IN CENTRAL ROMANIA

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Backgrounds. Several epidemiological data suggest the involvement of hepatitis C virus (HCV) in the pathogenesis of some B-cell non-Hodgkin's lymphomas in areas with high prevalence of HCV infection. Aims. To assess the prevalence of anti HCV antibodies in a cohort of 54 consecutive patients with B-cell non-Hodgkin's lymphoma admitted to the Department of Hematology of our center from October 1997 to December 2005 in the Sibiu County (a region in the center of Romania with 446,000 inhabitants). The control group was a cohort of 2,445 blood donors tested in the period of time from 1995 to 2002. Methods. In order to test the presence of anti HCV-antibodies in patients with B-cell NHL a third generation ELISA test was used. The two patients positive for HIV infection were excluded for this study. In the control group a second generation ELISA test was used, from 1995 to 1999 and a third generation one afterwards. Results. From the 54 patients with B-cell NHL (age between 22-77 years, male/female ratio 1,25) 13 were positive for anti-HCV antibodies (prevalence=24,07%). In the NHL HCV positive patients the male/female ratio was 0,625. In the blood donors control group, 108 were positive for anti-HCV antibodies (prevalence=1,28%). Conclusions. The prevalence of anti HCV antibodies was significantly higher in the cohort of B-cell NHL than in blood donors control group. From the 13 B-cell NHL tested positive for anti-HCV antibodies one was healthy carrier, 4 had liver cirrhosis and 8 had chronic hepatitis. We note 2 deaths with clinical and biochemical phenomena of liver failure (one case of B-cell NHL with hepatic cirrhosis and one case of B-cell NHL with chronic hepatitis) related to chemotherapy. Conclusions. 1. In the county of Sibiu we found a significantly higher prevalence of anti-HCV antibodies in B-cell NHL compared to blood donors control group, indicative of the fact that HCV may be involved in the etiopathoge-
Several studies described a variable incidence of infection of an organ. From 10 V. Matoska, J. Schwarz, It has been accepted that the hypermutation status of patients treated with alemtuzumab. No prospective reports currently provide results of oral valganciclovir as pre-emptive therapy in patients with CMV reactivation during alemtuzumab treatment. We explored the efficacy and safety of oral valganciclovir as a therapy of CMV reactivation and of prophylaxis of CMV disease. Methods. Starting from May 2004, we treated 10 patients (9 males and 1 female; median age 57). Six patients were in partial response after previous chemotherapy regimen containing fludarabine, and 4 were refractory to previous treatment (range 1-7). All patients received alemtuzumab at 10 mg as target dose, 3 times weekly for a prolonged period of 18 weeks. The drug was delivered subcutaneously and, in order to further minimise adverse local therapy-related effects and make the treatment more manageable, were associated with 50 mg of hydrocortisone s.c. for the first two weeks. At baseline all patients had undetectable CMV DNA but were positive by serology. Prophylaxis with oral acyclovir 800 mg bid was given during therapy and for a months after alemtuzumab therapy. CMV reactivation was detected weekly in peripheral blood mononuclear cells by PCR and was consid-
ered positive if >200 copie/mL. CMV disease was diagnosed from the cal evidence of CMV disease. CMV reactivation appeared after a medi-
ian of 5 weeks (range 4-6) of treatment. The alemtuzumab and acyclovir prophylaxis were discontinued and the patients were treated immediately with oral valganciclovir 900 mg bid. Only one patients required hospitalization for fever. After a median of 14 days (range 9-21) of antivi-
eral therapy all patients had achieved negative CMV PCR assays; oral valganciclovir was reduced at 450 mg bid and alemtuzumab treatment were resumed. No myelotoxicity or other side effects were observed during the treatment with oral valganciclovir. None of the 4 patient showed other episode of CMV reactivation after re-introduction of alem-
tuzumab. Conclusion. We successfully use valganciclovir in all patients with CMV reactivation. The response was prompt and there was no progression to CMV disease, no relevant clinical toxicity and unneces-
sary hospitalization for drug administration. Valganciclovir is effective and safe as CMV prophylaxis in CLL patients treated by alemtuzumab, allowing an easy management of a therapy previously difficult to be routinely used.
presented data would like to stress the necessity to identify/compile the most comprehensive IgVH database to be used for the determination of IgVH mutation status in CLL.

**1205**

**ALLOTRANSPLANTATION FOR CHRONIC LYMPHOCYTIC LEUKEMIA A SINGLE CENTRE EXPERIENCE IMPLYING ITS APPLICABILITY AND CURATIVE POTENTIAL**

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It is increasingly clear that autologous hematopoietic cell transplantation (alloHCT) offers currently the only curative option for chronic lymphocytic leukemia-CLL, but the relatively high transplant related mortality has limited its application. The recent experience following both the use of newer first line treatment with purine analogues and less toxic pre-transplant preparative regimens appeal for wider trials evaluating alloHCT early in the CLL course in younger patients. Materials. Ten patients (F/M=5/5), median age 44.5y (36-55), time from diagnosis to alloHCT 3 years (1-7.5). After diagnosis patients were treated using 1-5 different chemotherapies regiments, all obtained purine analogues and all displayed treatment resistant and progressive course. Other treatments included radiotherapy (n=2), anti-CD20 MoAb (n=2), anti-CD52 MoAb (n=1), anti-CD52 MoAb and allogeneic HCT (n=1). The disease status at alloHCT was as follows: CR = n=4, PRn=3, NRn=3. AlloHCT characteristics: HLA matched Sibling Donor HCT (n=8), HLA single allele mismatched SibHDT (n=1), matched Unrelated Donor HCT (n=1). Stem cell source for SibD transplant: bone marrow - 2, peripheral blood - 2 (two patients post-induction CD and CD34+ cells add back), BM+PB-1, for UHD-HCT - bone marrow in 1 pt. Conditioning: myeloabla- tive Ctx+TBI: n=2; Ctx+TBI+alemtuzumab: n=1; reduced intensity: alemtuzumab (20 mgx5)+fludarabine (80 mg/m² 2x5)+melphalan (140 mg/m²); n=7. The number of transplanted cells: nucleated cells 4,25x10⁸ (0.045-12); CD34⁺ cells 4,3x10⁶ (1.6-9.6); CD3⁺ cells 55x10⁶ (15-514) / kg recipient body weight. All transplantations were performed in intensive care, sterile HEPA units. GVHD prophylaxis consisted of cyclosporine A and methotrexate. Results. All patients engrafted. Hematopoietic recovery was as follows: granulocytes to 0.5 G/l - 22d (11-55); PLT to 50 G/l - 24d (13-40). One patient died on day 92 after transplantation of pulmonary Aspergillosis and hepatitis after LPD due to EBV infection transmitted from the donor. The remaining 9 patients achieved CR after transplantation. All 3 patients after myeloablative conditioning acquired full donor chimerism. Among RIC conditioned patients at 6 months 2 displayed full donor chimerism, 3 mixed chimerism and one presented autologous recovery. Acute GVHD grade I was observed in 8/10 patients, limited cGVHD in 3 patients and extensive cGVHD in 2. Six patients developed CMV reactivation, one VZV, and one HBV. Two patients (both after ablative conditioning) died due to late complications: on day 180 (cGVHD with obstructive bronchioli- tis) and on day 720 (chronic hepatitis). No patient relapsed with CLL suggesting efficacy of the mechanism. At 53 months after transplantation the probability of OS and DFS equals 60% with median observation time of 13 months (7-53). This observation compares well with recent other data (Toze CL et al 2005; 5y OS 59%) and suggests that allotransplantation offers an effective treatment with curative potential for progressive CLL patients who are in good biological condition.

**1206**

**SIGNIFICANCE OF SOME FACTORS IN THE ERA OF MODERN CLL THERAPY**

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**Background.** Expression of CD38, high level of Bcl-2 and B2-microglobulin and absence of CD95 expression are well-known unfavourable prognostic factors (UPF) for overall and progression free survival (OS and PFS), respectively. It is uncertain whether they retain their significan- ce in the time of fludarabin (F) and mabthera (Rituximab (R) ther- apy. Aim. To evaluate the influence of the above mentioned prognostic factors on clinical course of CLL in patients (pts) treated with modern therapy. Methods and patients. Sixty nine pts with B-CLL were included in this study (median age 59,5 years; Binet stage A - 1, B - 41, C - 27; median follow up was 143 mo, median follow up after the start of treat- ment was 48 mo). Thirty four pts received FC treatment - F 25 mg/m² and cyclophosphamide (Q 300 mg/m² for 5 days; 35 pts received RFC treatment - R 575 mg/m² on day 1, FC regimen on days 2-4. All pts received 6 cycles of therapy. The multivariate analysis with Cox’s regression model was used. Results. 18,5% of pts had all factors investigated, 27,7% had 3 and 21,5% 2 unfavourable factors in different combina- tions. One factor was found in 26,2% and none in 6,1%. In pts without UPF the OS was 107 mo in pts with 1-2 UPF, 57 mo with Bcl-2 and CD38 expression, 70 mo. High B2-microglobulin level as well as absence of CD95 expression had no prognostic significance. The multivariate analysis showed that expression of CD38 (Relative Risk RR=0,57, p=0,3) and high level of Bcl-2 (RR=0,61, p=0,8) had the most pronounced negative influence on OS. For PFS lack of CD95 expression (RR=0,83, p=0,099) and especially expression of CD38 (RR=1,26, p=0,059) were the most unfavourable factors. Median PFS was not achieved in pts with any UPF combinations without CD38 expression whereas in pts with all 4 UPF it was only 20 mo. Conclusion. Modern therapy with FC and RIC allows overcome the negative influence of high level of B2-microglobulin and Bcl-2 and lack of CD95 expression. CD38 expression retains its unfavourable significance.

**1207**

**INFLUENCE OF CLADRIBINE ON BONE MARROW ANGIOGENESIS IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS**

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**Backgrounds.** Angiogenesis is the process of formation of new blood ves- sels. The process is increased in many neoplastic diseases, including chronic lymphocytic leukemia (CLL). The nuclear side analogues, fludarabine and cladrabine, represent a novel group of cytotoxic agents with high activity in low grade lymphoid malignancies. Fludarabine decreases bone marrow vessels density in CLL patients. The influence of cladrabine on bone marrow angiogenesis in CLL was not studied so far.

Aims. The aim of the study was to evaluate the influence of cladrabine on angiogenesis in bone marrow of CLL patients. Methods. Parafin-embal- ded trephine biopsies were prepared and stained with antibody to CD34 for endothelial cells in patients with CLL before and after treatment with cladrabine. Number of microvessels were counted in hot spot places, the areas, with highest vessels density under the microscope in 200x magnification. Results. Trephine biopsies from 14 previously untreated progressive CLL patients were evaluated before and after treatment with cladrabine. Female/male ratio was 4/6 and median age of the patients 59 years (range 44-73). Staging according to Rai : Rai 0 2 patients, Rai 1-4 6 patients. All of the patients received cladrabine alone (4 patients), in combination with cyclophosphamide (7 patients) or in combination with cyclophosphamide and mitoxantron (3 patients). All of the patients responded to the therapy and were in complete remission (4 patients) or partial remission (10 patients) according to NCI sponsored Working Group criteria. Median vessels number in hot spots places before treatment was 105 (range 45-238) and after treatment 65 (range 35-1600, p=0,02). There were no differences between different regimens containing cladrabine. Conclusions. Number of vessels in bone marrow of CLL patients was decreased after treatment with cladrabine containing regimens.

**1208**

**MATURE B-CELL AND T-CELL NEOPLASMS PRESENTING WITH LYMPHOCYTOSIS: A SYSTEMATIC DIAGNOSTIC APPROACH BASED ON CLINICAL, MORPHOLOGIC, IMMUNOPHENOTYPIC AND PATHOLOGICAL FEATURES IN 373 CONSECUTIVE CASES**

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**Background.** Expression of CD38, high level of Bcl-2 and B2-microglobulin and absence of CD95 expression are well-known unfavourable prognostic factors for overall and progression free survival (OS and PFS), respectively. It is uncertain whether they retain their significan- ce in the time of fludarabin (F) and mabthera (Rituximab (R) ther- apy. Aim. To evaluate the influence of the above mentioned prognostic factors on clinical course of CLL in patients (pts) treated with modern therapy. Methods and patients. Sixty nine pts with B-CLL were included in this study (median age 59,5 years; Binet stage A - 1, B - 41, C - 27; median follow up was 143 mo, median follow up after the start of treat-
lymphocytosis, focusing in particular on the differential diagnosis of clonal B-cell lymphocytoses. Methods. Between January 2003 and December 2004, we evaluated 373 consecutive patients (M/F: 190/183) with an absolute (i.e. >5000/m$^3$) (81%) or relative (19%) lymphocytosis. Median age was 68 years (range: 19-91). Clinical features, lymphocyte morphology, immunophenotype, BM and/or lymphode histopathology were reviewed. The study was independently reviewed by three experts. Immunophenotype was performed on fresh whole blood samples using four-color immunostaining with a panel of B- and T-cell markers, a FACSCalibur flow cytometer (BD Biosciences) and the Cell Quest software (BD Biosciences). T-cell clonality was assessed by PCR analysis of the T-cell receptor (TCR) $\gamma$ chain variable regions and using the TCR V$\beta$ Kit (Beckman-Coulter Immunotech, Marseille, France) for the TCR$\beta$ chain families repertoire. Histopathology evaluation of BM and/or lymphode was performed in cases whose PB morphology and immunophenotype suggested a likely diagnosis of B/T non-Hodgkin lymphoma (NHL) or could not distinguish between B-NHL/chronic lymphocytic leukemia (CLL). Results. A B-lineage lymphocytosis was recorded in 81% cases (n=301; 241 CD5+ and 60 CD5-), a T-lineage in 14% (n=52) and a normal lymphocyte pattern in 5% (n=20). In terms of PB morphologic/immunophenotypic criteria, among clonal B-lymphocytoses, 44.5% (n=154) had features of CLL, 44.5% (n=134) were B-NHL, 10% (n=30) were provisionally defined as B-NHL/CLL for intermediate features, 1% (n=3) were hairy cell leukemia (HCL). Of 107 CD5+ cases not fulfilling the standard diagnosis of CLL, 59 underwent BM and/or lymphode biopsy; 41% were diagnosed as B-NHL with leuemic spillover (5 marginal zone lymphomas (MZL), 4 mantle cell lymphomas, 2 follicular lymphomas (FL), 2 lymphoplasmacytic lymphomas (LPL), 11 unspecified low-grade B-NHL) and 59% with CLL. Even among CD5+-CD25+ cases not fulfilling the standard diagnosis of CLL, 25% (11/43) proved to be leukemic B-NHL at histopathology evaluation. Of 60 CD5- cases, 37 underwent a biopsy; the final diagnosis was MZL in 21 cases, FL in 4, LPL in 3, HCL in 2, unspecified low grade B-NHL in 7. In CD5+ B-NHL and CD5- B-NHL, the expression of CD38 (p<0.001) and adhesion molecules (CD11a, CD18) (p<0.001) were significantly higher than in CLL, as well as the presence of superficial adenopathy (p<0.001), splenomegaly (p<0.001), thrombocytopenia (p=0.05) and raised LDH (p<0.001). Among T-NK lymphocytoses, 43 cases showed a T-LGL expansion (reactive in 21, clonal in 22, 2 NK-LGL, 3 T-NHL, 4 unspecified) cell infiltrate with characteristic features. Conclusion. This study highlights the frequency of various B- and T-cell neoplasms presenting as lymphocytosis and the value of lymphocyte morphology, immunophenotype and histopathology in identifying and subclassifying low grade B-NHL with leukemic presentation. These observations have prognostic-therapeutic implications.
bone pain and fractures. Aims. Aim of this study has been to evaluate the efficiency of ibandronate (Bondronat, F.Hoffman-La Roche) on the course of bone lesions in myeloma patients and also its safety particularly concerning renal function. Methods. We analysed a group of 28 patients in clinical stage III-A or III-B (median age 59.9 years, range 42-77, male: female ratio 13:15) who were currently treated, independent-ly from the adopted chemotherapy, with Bondronat given as a IV infusion or by mouth from four weeks before the first treatment, is feasible and may induce a new significant response. The ONJ in patients with MM who underwent dental or oral surgery after discontinuing zoledronic acid several months before. All patients received treatment with chlorhexidine rinses, antibiotics and surgical debridement. The follow-up after the diagnosis of ONJ was at least six months. Conclusions. The ONJ in patients with MM who underwent dental or oral surgery appears to be associated with long term exposure to zoledronic acid. A previous dental pathology and the time of exposure to zoledronic acid are main factors in the development of the ONJ. The long-lasting bone effect of bisphosphonate could explain the appearance of osteonecrotic lesions after discontinuing treatment with bisphosphonate.

1213 RE-TREATMENT WITH BORTEZOMIB IN MULTIPLE MYELOMA
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Background and Aims. Bortezomib is an effective agent for multiple myeloma, currently licensed for the treatment of relapsed/refractory disease after first line therapy. There are, however, few reports about the use of bortezomib as re-treatment (re-challenge) in myeloma patients who have previously received the same drug for their disease. The clinical outcome of five patients with these characteristics is reported. Methods. Five patients were re-treated with bortezomib alone or in combination; three were male and two female, with an age ranging from 45 to 66. All patients had a prior salvage therapy with bortezomib or borte-zombib plus dexamethasone, after 2 to 4 lines of other chemotherapies, including autologous stem cell transplantation and thalidomide. All patients achieved at least a partial response (reduction of M-component >50%) after the first treatment with bortezomib and relapsed after 3 to 19 months. Results. Bortezomib was re-administered at the standard dose of 1.5 mg/m² IV, days 1, 4, 8, 11, q2wks in four patients; dexamethasone 20 mg/m² for 2 days after each infusion of bortezomib was added in 3 out of 4 patients. Severe hematological or extra-hematological toxicities did not occur, but dose reduction or temporary interrup-tion of the treatment were occasionally required, mainly due to moderate thrombocytopenia, neuropathy and skin rashes. After 4-8 cycles, three patients achieved a partial response, with reduction of M-component >50% and concomitant consistent decrease of marrow plasma cell infiltration. Duration of second response to bortezomib ranged from 3 to 8 months. In one patient a stabilization of the disease was obtained. In the fifth patient bortezomib was employed at the dose of 1.3 mg/m² days 1 and 4 in combination with melphalan (100 mg/m² e.v.), thalido-mide (100 mg/d for 5 days) and dexamethasone (40 mg e.v. for 4 days), who had conditioning regimen (MVTD) for a further autologous stem cell transplantation. An impressive, rapid complete response with negative serum immunofixation (M-component was 6.1 g/dl before transplant) occurred. This response had a brief length and the patient relapsed after 3 months. The same regimen was given once again and the same complete response was achieved in a few days. The patient, however, died of interstitial pneumonia during the aplastic phase of transplant. Conclu-sions. Our data, although limited, suggest that re-treatment with borte-zombib of myeloma patients, who experienced a clinical benefit after the first treatment, is feasible and may induce a new significant response.

1214 A SINGLE CENTER REPORT ON AUTOLGOUSTEM CELL TRANSPLANTATION FOR PATIENTS WITH MULTIPLE MYELOMA
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Autologous stem cell transplantation (ASCT) is a recommended treatment option for patients with advanced multiple myeloma (MM). From Oct. 1996 to Aug. 2005 we performed 121 ASCT in 71 MM-patients (age 56 median:37-69) years; female:31, male: 40). Thirtythree patients (pts) underwent transplant with a single course (20 pts. between 1996-1999 and 7 pts. thereafter due to ineligibility for a second transplant (e.g. late infections (2), toxic side effects: cardiotoxicity (2), neurotoxicity (1), dermatitis (1), sMDS (1)). Thirteeneighty patients underwent multiple cours-
es of transplantation (26 double and 12 triple ASCT). No significant differences between both groups were seen according to age, sex or stage of disease at the time of ASCT, but more patients who relapsed after conventional treatment (15 vs. 5 pts) were included in the single than in the multiple ASCT group. Conditioning chemotherapy consisted of Melphalan 200 mg/m² for single and double ASCT and 100 mg/m² for triple ASCT. Autografting was performed with peripheral blood stem cells. Renal failure was contributing to 4/12 early deaths. Bacterial infection directly caused 11/12 early deaths (91%). Specifically pneumonia occurred in 42 (30%) of 136 bacterial infections, 12 (9%) of fungal infections, 4 (3%) of viral infections and 1 (1%) of protozoal infections. Hospital mortality was further defined as death within 60 days. Infectious diseases were confirmed clinically or microbiologically. Secondary endpoint was to assess the response rate. It was estimated based on the best response to therapy for each patient during the course of treatment. Statistical analyses were performed using SPSS version 13.0. Fisher’s Exact test was used to evaluate the statistical significance of associations in two-way contingency tables. A p-value < 0.05 was considered as statistically significant. Results. Between January 1995 and December 2005, we treated 122 patients with multiple myeloma, 67 patients received VAD (54.9%), 35 patients received thalidomide and dexamethasone (28.7%) and 20 patients reached melphalan/prednisone (15%). The frequency of patients in the CR, VGPR or NCR group of thalidomide and dexamethasone was 80% (CR22.8%, VGPR/NCR 20% and PR, 37.2%) being higher than VAD, 50% (CR 16.4%, VGPR/NCR 5.9%, PR 28.4%). p < 0.0005 Early mortality before day 60 occurred in 12 (9.8%) of patients registered onto MM database at our Institution from 1995 to 2005. Patients who died early were shown to be older (> 65 years; p < 0.0001), have a poorer performance status (p < 0.0001), and reduced serum albumin (p < 0.04) compared with the remainder of the MM database. Early mortality was associated with a greater tumor burden and activity (creatinine, β2-microglobulin, albumin, hypercalcaemia and C-reactive protein; p < 0.0001). There was a significant correlation between early death (ED) and evidence of hematopoietic dysfunction as evidenced by anemia (p < 0.001), thrombocytopenia (p < 0.0001), neutropenia (p < 0.04), and lymphocytopenia (p < 0.007). Renal function was impaired in twice as many of the early death patients with higher presentation serum creatinine and urea (p < 0.005). Renal failure was contributory to 4/12 early deaths. Bacterial infection directly caused 11/12 early deaths (91%). Specifically pneumonia occurred in 42 (50%) of 156 bacterial infections (122 patients), and 11/12 patients in the ED group. Generalized sepsis occurred in 18 (15%) of 136 bacterial infections, and other infections occurred in 52 (58% Urinary tract infections) and 24 patients (18%) (eg. osteomyelitis, peritonitis and meningitis). Conclusions. This study describes the complications and related mortality that occur soon after diagnosis of myeloma is made. Measures to prevent infectious complications has been described previously. In addition, reduction of renal toxicity also has to be mandatory.

1217
INTERNATIONAL STAGING SYSTEM FOR MULTIPLE MYELOMA IN A MEXICAN POPULATION
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Background. Studies conducted in the 1960’s and early 1970’s identified a number of clinical and laboratory parameters that are independent predictors of survival duration. In 1975, Durie and Salmon introduced a staging system that predicted myeloma cell tumor burden. Factors in the DS classification included the level and type of monoclonal protein, hemoglobin, calcium level, and number of bone lesions. Creative line (Substage A: serum creatinine < 2 mg/dl; and substage B: serum creatinine > 2 mg/dl) further defined lower versus higher risk patients in each tumor mass stages. Recently Greipp et al, reported the International Staging System (ISS) as a simple and reliable staging system for multiple myeloma. The ISS system was validated by demonstrating effectiveness in patients in North America, Europe and Asia but not in Central America. Aims. The main objective of our study was to assess the effectiveness of ISS in a mexican population. We also reported the outcomes in terms of overall response according to each group of treatment and ISS was compared with the DS staging system.
Patients, material and methods. We enrolled all patients who fulfilled the criteria for multiple myeloma between January 1995 and December 2005. The present study is a retrospective, descriptive, longitudinal and observational one. All patients had survival status and date of last follow-up recorded within 6 months of the data analysis. At the time of analysis, 54.1% of patients had died. Statistical analysis.Univariate and multivariate survival analysis. The variables are ranked by hazard ratio, with all being significant at the p<0.001 level.Fisher's Exact test was used to evaluate the statistical significance of associations in two-way contingency tables. A p value <0.05 was considered as statistically significant. Results. One hundred and twenty two newly diagnosed multiple myeloma patients were evaluated, 76 (62.3%) male and 46 (37.7%) female, aged 46-85 (median 64). Compared with the DS classification, ISS provides a simple reliable classification of patients. ISS stage I is underrepresented, maybe because stage I patients usually are asymptomatic or not included in clinical trials. B2 microglobulin greater than 5.5 mg/dl appeared to be the most highly statistically significant result (p 0.005). Median survivals were as follows: I, 74 months; II, 48 months and III, 20 months (p 0.0002 for differences). We also evaluated outcomes in terms of survival; creatinine > 2 mg/dl, p <0.03, platelet count less than 130,000, p<0.005 , CRP >6 mg/L p 0.003 , albumin < 35 g/L (p 0.005) and cyto genetics abnormalities such as del 13 , resulted in worst overall survival. We reported an increased mortality in those patients with 14q32 abnormalities or deletion 13 (p 0.003).Conclusion. We found that B2 microglobulin higher than 5.5 mg/L is the best cut off to discriminate survival in newly diagnosed MM patients. Based on this result the following question is mandatory; why are serum B2MG and serum albumin such powerful prognostic factors? Serum B2MG reflects not only tumor mass and renal function but also other as yet unknown parameters, possibly including immune function.

1218
DIFFERENCE BETWEEN MALE AND FEMALE PATIENTS WITH MULTIPLE MYELOMA ON LIPID PROFILE
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Purpose: The aim of this study was to investigate the difference between male and female patients with multiple myeloma on lipid profile.

Material and Methods. 77 inpatients with multiple myeloma, aged 73±7, 42 females (F) and 35 males (M) were studied. Serum protein electrophoresis and immunoelectro phoresis (IgG, IgA, IgM, and IgE) and plasma lipid levels as Total Cholesterol (TC), Triglycerides (TG), HDL and LDL, from all patients were measured and compared between females and males. All patients belong to our Internal Medicine Clinic and results were analyzed in the same laboratory.

Results. Electrophoresis (%): Albumin 45.6±9.5, 50.6±6.2(M) - 39.6±9.1(F), p=0.21, β1 globulin 3.3±1.2, 5.7±1.8(M) - 3.2±1.6(F), p=0.62, β2 globulin 10.7±4.3, 14.2±1.9(M) - 8.8±3.7(F), p=0.57, β globulin 13.1±8.3, 10.6±3.6(M) - 14.4±9.8(F), p=0.16, α globulin 29.8±16.4, 21.6±11.6(M) - 34.2±17.8(F), p=0.27, Immuno electrophoresis (mg/dl): IgG 2439±2636, 2999±3195(M) - 1449±518(F), p=0.003, IgA 99±66, 56±71(M) - 60±95(F), p=0.63, IgE 28±674, 61±26(M) - 507±926(F), p=0.02, Light chains (mg/L): Ig Light 8.5±10.0, 12.1±10.8(M) - 1.5±3.9(F), p=0.004, Ig Light 2.9±3.7, 1.2±2.9(M) - 5.9±4.3(F), p=0.02. Lipid profile (mg/dl): TC 173±54, 147±81(M) - 183±55(F), p=0.000, TC 154±69, 108±62(M) - 175±62(F), p=0.04, HDL 42±12, 35±17(M) - 45±9(F), p=0.01 and LDL 95±42, 75±57(M) - 102±32(F), p=0.02. Conclusion. The study shows that in patients with multiple myeloma the TC, HDL and LDL are increased statistically significant in females, while the level TG between female and male have not statistically significant.

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VELCADE AS AN ACTIVE AGENT FOR MULTIPLE MYELOMA PATIENTS. EXPERIENCE OF A SINGLE CENTER
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Velcade (PS-341, Bortezemib), a proteasome inhibitor, has been shown to be efficient in the treatment of relapsed and refractory Multiple Myeloma (MM) and approved for this indication. Aims. We report the results of a nearly 3-year experience regarding the use of this drug in this subset of patients followed in our center. Between April 2003 and February 2006 we treated 27 patients with MM. Patient population consists of 15 males and 12 females, with a median age of 55 years (range 32-76). 17 were IgG, 9 IgA, 1 light chain. All patients were in stage III of disease with a median time of observation (from diagnosis}

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to Velcade therapy) of 54 months (range 11-120), pretreated at least with two lines of therapies and were refractory or in relapse after the last treatment. Thirteen patients had undergone autologous transplantation and 2 allogeneic one, with a median number of previous therapeutic lines of 2 (range 1-5). Velcade 1.3 mg/m² was administered on day 1, 4, 8 and 11 of a 21-day treatment cycle for 5 cycles according to the tolerability in an inpatient hospital regime. A median of 6 cycles (range 2-8) were administered to the overall population. Thirteen patients concluded their program and 14 discontinued the treatment: 1 because received allogenic stem cell transplantation, 8 for adverse events and 5 for progression of disease. In this heavily pretreated population our primary end point was to obtain a decline in Monoclonal Component (MC) of at least 25%. All patients but 2 were considered evaluable for response because treated at least with 3 cycles of therapy. Thirteen patients responded to treatment: 7 (28%) achieved a reduction of MC level >75%, 4 (16%) <75% and > 50% and 2 (8%) < 50% and > 25%. Twelve (48%) showed no response. The median number of cycles to achieve a response was 3 (range 1-8). After a median time of observation of 28 months (range 5-54) the median duration of response was 7 months with 5 patients still in response, 8 relapsed and 4 of them died for progression of disease. Among the 12 (48%) not responding patients 3 died. The majority of adverse events, resolved with the discontinuation of treatment, were nausea, vomiting, diarrhea, fatigue, thrombocytopenia, peripheral neuropathy, and constipation. Very poor prognosis of our patients, this study adds further evidence concerning the efficacy of this new drug. Velcade can be considerate an effective anti-myeloma drug even though its toxicity must be taken into account in designing new clinical trials.

1221
BORTEZOMIB WITH OR WITHOUT DEXAMETHASONE IN HEAVILY PRETREATED MYELOMA PATIENTS: PRELIMINARY SAFETY AND ACTIVITY PROFILE FROM A SINGLE CENTRE
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Background/Aims. Patients with multiple myeloma respond to front-line chemotherapy but relapse is virtually inevitable and response duration decreases with each salvage regimen. A proteasome inhibitor, is approved for the treatment of relapsed myeloma, while addition of dexamethasone may result in enhanced tumour control. We evaluated the activity and safety of bortezomib with or without dexamethasone in 20 pretreated myeloma patients. Methods. 20 heavily pretreated myeloma patients (median number of previous therapies 4), with a median age of 73 years (range 54-82), received bortezomib 1.3 mg/m² intravenously on days 1, 4, 8 and 11 of a 21-day cycle for eight cycles. When it was combined with dexamethasone, this was given on days 1,2 of each cycle at dose 20mg daily. Bortezomib was withheld if grade 3 toxicity occurred, the dose reduced to 1 mg/m² or 0.7mg/m² in the event of drug-related grade 3 non-haematological toxicity (WHO toxicity criteria). Response evaluation according to EORTC criteria was performed every two cycles. 12/20 patients received the combination of bortezomib with dexamethasone and 8 bortezomib monotherapy when contraindication for steroid administration existed. Results. No patient received eight cycles of treatment due to toxicity. The median number of cycles administered was 4 (range 1-6). The toxic event most frequently responsible for therapy withdrawal was grade 3 peripheral neuropathy. Thirteen cases of grade 3 peripheral neuropathy were observed. Thrombocytopenia was the most frequent adverse event (3 cases of grade 4 and 9 of grade 3) but no severe hemorrhagic episode took place. Three patients developed grade 4 paralytic ileus and 3 from treatment interruption. With a median follow-up of 11 months, 17 patients had a response (1CR - 16 PR), while three patients had refractory disease. 14 of 20 patients are alive and 7 out of 20 in remission. The median time to progression was 12 months and the 1-year progression free survival was 54% (8.4-15.6 months). For the monotherapy group the median time to progression was 10 months whereas for the combination dexamethasone - bortezomib 12 months, a difference not significant (Log rank 2-sided p=0.72). Among responders the median duration of response was 7 months (range 3-12). For the monotherapy and combined treatment groups the median duration of response was 5 and 7.5 months respectively (Student t-test p=0.65). The 1-year overall survival is 80% with the median overall survival not reached yet. Conclusion. We report evidence of satisfactory activity of bortezomib/dexamethasone in this group of 20 heavily pretreated patients with advanced myeloma. Peripheral neuropathy was unexpectedly a major problem in a patient cohort pretreated with nerve-damaging therapies such as VAD and thalidomide. Neurologic toxicity caused reduction of dose-intensity and bortezomib discontinuation, factors aggravating the overall antitumour effect. Research efforts towards modulation of neurotoxicity and optimisation of bortezomib schedules may pave the way for enhanced myeloma control.

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LEUKOCYTE ALKALINE PHOSPHATASE SCORE IN MULTIPLE MYELOMA; CORRELATION WITH G-CSF, IL-6 AND TNF-α
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Backgrounds. The leukocyte alkaline phosphatase (LAP) score has been reported to be elevated in patients with multiple myeloma (MM), but its clinical significance has not been clarified. Some authors reported that interleukin-1α, IL-6 and G-CSF genes were co-expressed in most patients with MM. Aim: In the present study, we determined the LAP scores of peripheral blood neutrophils and the serum levels of G-CSF, IL-6 and tumor necrosis factor-α (TNF-α) in patients with MM at diagnosis, and healthy controls and made the respective correlations. Material and Methods. We examined 25 patients with MM, aged 62±10, at diagnosis and 10 normal subjects, age 45±5. The LAP score was examined by using naphthol AS-Bl phosphate (Sigma Chemical St.Louis,MO). The serum levels of G-CSF, IL-6 and TNF-α were measured by a sandwich enzyme immunoassay test (R&D Systems,Minneapolis, MN, USA) and measured in a Microplate Reader Serio-Brio™ (Radim Group). Results. The mean LAP scores of patients with MM vs control subjects were 295±58 and 187±46 respectively. The mean value of LAP in MM patients is significantly higher (p<0.001). The serum TNF-α levels of the patients with MM vs those of controls were 15.2±12.4 pg/mL and 3.8±2.9 pg/mL. The mean value of G-CSF in MM patients was significantly higher than the control group (p<0.01). The serum levels of IL-6 in MM patients was 6.7±13.2 pg/mL while in the control group it was under the minimal detectable level. The serum TNF-α levels of the patients with MM were 3.9±6.5 pg/mL vs 0.02±0.19 pg/mL of the control subjects, showing a significant higher mean level in the MM patients vs the normal subjects (p<0.05). The correlation coefficients between the LAP score and the serum levels of G-CSF, IL-6 and TNF-α were 0.450 (p<0.001), 0.270 (p<0.05) and 0.380 (p<0.01). Conclusion. The LAP score and the concentrations of TNF-α and IL-6 are significantly higher in MM patients vs normal subjects. The most significant correlation was noted between the LAP scores and the G-CSF level. This finding suggests that the increase of the LAP score in MM may reflect a stimulation of the neutrophils by G-CSF.

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PROGNOSTIC FACTORS AFTER FIRST COURSE VAD (VINCRISTINE, DOXORUBICIN, DEXAMETHASONE) IN MULTIPLE MYELOMA PATIENTS TREATING FOLLOWING ASCC
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Backgrounds. High-dose melphalan with peripheral blood stem cell rescue represents today the standard therapy for young multiple myeloma (MM) patients. The most frequent therapy inducing remission is chemotherapy according to VAD protocol. Aims. The aim of this study was to determine prognostic factors and overall survival (OS) according to results of first VAD chemotherapy. Materials and methods: The study group consisted of 64 MM patients (32M/32F), median age 57.5 y; range 35-75y. Diagnosis was established on the base of common rules. Patients were in the stage of the clinical progression of the disease on the serum level of k- or λ- M-protein and monoclonal protein IgG class was observed in the serum at 36 cases of the patients, IgA-11, IgD-1, Bence Jones-15 and nonsecretory MM-1. Patients achieved following types of chemotherapy: 64 pts received chemotherapy according to VAD with following auto-PBSCT. We divided pts in 2 groups: first group: Pts, who are died; OS <740 days (medi-an OS-740 days), second group: Pts, who are living; OS >740 days. We compared results of investigations/studies, which are made to recognise MM before and after first VAD course. All of the results have been statistically tested by using 7-student test for the independent groups. For statistically significant results were p<0.05. Results. The baseline serum concentration of creatinine was statistically significant (p=0.05) larger in first group (mean: 4.6 mg/dl ±4.17; range: 0.59-14.36 mg/dL) according to the second one (mean: 1.01 mg/dL ±2.34; range: 0.66-11.3 mg/dL). We detected statistically significant results (p<0.05): -decrease in baseline concentration of monoclonal protein (mean: in first group from 5.5 g/dL (range: 0.86-15.85 g/dL ±3.8) to 4.4 g/dL (range: 0.6-10.5 g/dL ±3.1); in
second one (mean) from 4.7 g/dL (range: 0.6-9.3 g/dL; +2.5) to 3.0 g/L (range: 0.6-9.1 g/dL; +2.2), reduction of β2-microglobulin in the second group from mean: 8 mg/L (range: 0.46-67.7 mg/L; +13.65) to 6 mg/L (range: 0.98-67.7 mg/L; +13.28); differences in: baseline 24 hour urine calcium between groups: mean: 3.24 mmol (range: 0.16-18.5 mmol; +8.3)-in first group according to: 6.5 mmol (range: 0.31-15.5 mmol; +7.5); value of decrease of 24 hour urine calcium between groups: mean: 1.0 mmol (range: 4 mg/m²×10^{-6} to 20 mg/m²×10^{-6}) in first group, mean: 7.2 mg/m²×10^{-6} (range: 0.31-33.3 mmol; +8.6)-in second one; an increase in 24 hour urine protein in first group (mean): from 2.7 g/L (range: 0.03-8.2 +2.44) to 4.32 g/L (range: 0.15-20.14; +6.8) and a decrease (mean)-in second group: from 1.3 g/L (range: 0.9-2.2) to 0.25 g/L (range: 0.14 +0.3); difference in 24 hour urine protein after first VAD chemotherapy between first and second group was 0.2 mg/m²×10^{-6} (range: -1.25 to 1.5)-in first group (mean): time to progression: 43 vs 380 days; time to new chemotherapy: 52 vs 475 days; OES: 300 vs 1145 days. Conclusion. Prognostic factors, which are determined OS in pts receiving VAD chemotherapy can be: 1: Reduction of mononuclear protein after first VAD course. 2. Reduction of β2-microglobulin after first VAD course. 3. Decrease in 24 hour urine protein and calcium after first VAD chemotherapy.

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RENTAL SAFETY AND PHARMACOKINETICS OF IBANDRONATE IN MULTIPLE MYELOMA PATIENTS WITH PRE-EXISTING RENAL INSUFFICIENCY

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Backgrounds. As multiple myeloma progresses, patients are at increasing risk of skeletal complications and renal deterioration. Bisphosphonates are the standard of care for the treatment of skeletal complications due to bone metastases from multiple myeloma and other malignancies. Clinical studies have shown that some intravenous bisphosphonates are associated with dose- and time-related declines in renal function. Ibandronate, a non-alkaline bisphosphonate, is indicated for use in patients with bone metastases from multiple myeloma and other malignancies.

Aims. In this open-label study we assessed the pharmacokinetics and safety of intravenous ibandronate 6mg in patients (n=40) with multiple myeloma and pre-existing renal insufficiency. Methods. Renal function deterioration was graded at baseline depending on creatinine clearance (grade 0: >80, 1: 50-79, 2: 30-49, 3: <30 mL/min). Patients received intravenous ibandronate 6mg (60 minute infusion). Ibandronate excretion and serum levels were measured over 48 hours. To minimize error, creatinine clearance was calculated using three methods (direct serum/urine measurements, Cockcroft and Gault formula and Modification of Diet in Renal Disease Study Group [MDRD]; Ann Intern Med 1999;130:461-70). AUC of serum ibandronate levels and ibandronate clearance were calculated. Markers of tubular damage, β-glutathione-S-transferase [BGST] and ß-N-acetyl-glucosaminidase [NAG], were measured at baseline, and at 24 and 72 hours following ibandronate infusion. Results. At baseline ten patients had normal renal function (stage 0), 30 had various stages of renal insufficiency. The remaining thirty patients had varying degrees of renal insufficiency, with ten evaluated as stage 3. There was a positive correlation between ibandronate elimination and creatinine clearance (r=0.87; p<0.00001). Total body clearance of ibandronate did not change significantly with renal insufficiency. The renal clearance of ibandronate was strongly correlated with renal clearance (r=0.87; p<0.0001). The AUC for ibandronate was not significantly different between other grades of renal function. Serum creatinine and urinary enzymes of tubular damage did not change significantly within 72 hours of ibandronate infusion. No acute nephrotoxicity was seen throughout the study. Summary and Conclusions. In this study, the elimination of ibandronate did not change significantly with renal function, while renal clearance, AUC and renal excretion of ibandronate serum levels significantly increased for patients with the most advanced renal deterioration. Due to its renal safety profile in phase III clinical trials, ibandronate is indicated in patients with mild-to-moderate renal impairment without dose adjustment. Ibandronate is the only bisphosphonate that is recommended for use in patients with severe (grade 3) renal insufficiency (ibandronate 2 mg). We conclude that a dose reduction is not necessary to maintain renal health. Despite renal function already being compromised in this patient group there was no evidence of acute nephrotoxicity with ibandronate. These data suggest that ibandronate may be suitable for use in multiple myeloma patients with or without pre-existing renal impairment without the need to reduce the dosage.

1225

RIES IN SERUM ALKALINE PHOSPHATASE (AP) ARE NOT CORRELATED WITH RESPONSE TO VELCADE (BORTezOMIB)

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Backgrounds. Velcade, a proteasome inhibitor, is a novel agent in the treatment of multiple myeloma (MM), showing promising activity even in relapsed/refractory MM. Recently Zangari and colleagues repeatedly claimed a clear correlation between rise in serum AP and activity of Velcade (abstract Sydney ’05, oral presentation ASH ’05 and Brit J of Haematol ’05). Serum AP could be a cheap and easy test to decide on an expensive treatment. We have/had a different impression and studied our myeloma population treated with Velcade, partly in a retrospective, partly in a prospective way. Methods. Between March ’03 and August ’05 32 evaluable patients were treated with Velcade for relapsed/refractory MM at our institution. 4 Patients presented with a light chain κ, 3 with light chain λ, 5 with IgκA, 2 with IgκA, 12 with IgκG, 7 with IgλA, and finally 1 with a non secreting MM. The youngest patient was 53 years, the oldest 85; 19 were male, 13 female. Prior to Velcade, they were treated with a mean of 3 lines of therapy (1-8); 16 underwent at least one stem cell transplantation. Results. The best responses to bortezomib were: 5 nCR, 14 PR, 8 MR, 4 SD and 6 PD (EBMT response criteria). We checked serum AP at baseline and after 6 weeks (strongest predictive value in the study of Zangari). We detected a rise in AP (64.1%) in all groups of response - even in progressive disease - indeed suggesting an osteoblastic activity, but no correlation with the type of response. Conclusions. 1. Velcade probably has an osteoblastic activity (besides other mechanisms of action), reflected in rises in AP. 2. In contradiction with a recent publication of Zangari et al., there is no correlation between myeloma response and level of AP increase.

1226

A SINGLE FIXED DOSE OF PEG-FILGRASTIM ALLOWS ADEQUATE STEM CELL MOBILISATION IN MULTIPLE MYELOMA PATIENTS

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Backgrounds. Autologous transplant is the standard of care for multiple myeloma (MM) patients aged less than 65 years. An adequate mobilization of stem cells is therefore essential to complete the therapeutic program. PEG-filgrastim (PEG-F), a long lasting conjugated form of filgrastim used as a single dose of 6 mg to shorten chemotherapy induced neutropenia, has been recently evaluated for stem cells mobilization in haematologic malignancies. Preliminary reports show that PEG-F is as effective as filgrastim in mobilizing MM patients with a better compliance. Aims. To evaluate the mobilization capacity and the safety of PEG-F after DCEP regimen in MM patients enrolled in a high dose program. Methods. We mobilized 11 previously untreated MM patients with a combination of DCEP chemotherapy (Decadron 40 mg/day i.v. days 1-4, Cyclophosphamide 700 mg/m²/day i.v. days 1-2, Etoposide 100 mg/scm/day i.v. days 1-2, Cis-Platin 25 mg/m²/day i.v. days 1-2) followed by a single subcutaneous dose of PEG-F 6 mg 48 hours after the end of chemotherapy. The first leukapheresis was performed when peripheral CD34+ cells were >20×10^6 and continued until at least 4×10^6/kg CD34+ cells were collected. Patients collecting <2×10^6/kg CD34+ cells were considered poor mobilizers. Results. The median number of CD34+ cells collected with 1 (6 patients) or 2 leukaphereses (5 patients) was 5.9×10^6/kg (range: 1.5-29.4). Nine patients mobilized 10 days after the end of DCEP therapy, 1 patient after 9 days, 1 patient after 11 days. One patient who had failed the first mobilization with filgrastim, with PEG-F collected the third leukapheresis and two leukaphereses. One patient did not mobilize (1/11: 9%). Median peak number of peripheral CD34+ cells at the time of collection was 60/mL (range: 24-418). Five patients showed WHO grade 3-4 therapy-related neutropenia and 2 WHO grade 2 throm-
bocytopenia. No patient experienced fever or infections or required transfusions. The majority of the patients complained of mild to moderate back pain easily controlled by oral analgesics. Conclusions. This study shows that a single fixed dose (6 mg s.c.) of PEG is safe and effective to mobilize adequate number of CD34+ cells in the majority of myeloma patients, a category usually considered worse mobiliser than patients with other hematological malignancies. In addition, the single administration of PEG shows better compliance than repetitive doses of filgrastim.

1227 EXANTHEMA AND HERPES ZOSTER INFECTION DURING VELCADE USE INCIDENCE, TREATMENT AND PROFYLAXIS
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Backgrounds. Bortezomib has been shown to be highly effective in the treatment of relapsed multiple myeloma (MM). Data from clinical trials show that the incidence of herpes zoster during bortezomib therapy is about 15%. Skin rash is quite common toxicity seen in MM patients treated with bortezomib. Where reported, its incidence in clinical trials ranged from 8 to 18%. We reported our results and treatment of this two adverse effects of bortezomib treatment. Methods. From December 2004 we treated 48 relapsed MM patients with bortezomib. Patients were treated with standard dosage schedule (intravenous infusions of bortezomib 1.3 mg/m² on days 1, 4, 8, and 11 of a 21-day cycle). Results. Our first two patients treated with bortezomib did not receive varicella-zoster virus (VZV) prophylaxis and herpes zoster developed in three of those patients (63%). Clinical manifestation of herpes zoster was typical, starting with itching and pain and exanthema appearing later. All patients were treated for the 2nd relapse of MM. Herpes zoster developed in the first patient during the 3rd cycle, in second patient during 6th cycle and in third patient during the 8th cycle of bortezomib. Therapy with bortezomib was subsequently interrupted and all three patients received treatment with acyclovir intravenously. Based on experience, we started to use prophylaxis with acyclovir 400 mg per os 3 times daily during bortezomib therapy. We did not note any VZV reactivations in 56 consecutive patient receiving VZV prophylaxis. This group also included five patients who already had VZV reactivation before bortezomib treatment. In 12 of 48 patients (25%), rash developed during the second treatment cycle. T he first cycle of bortezomib was well tolerated in all cases. Skin biopsy was done in first three patients , in all cases p erversal lymphoid infiltrates were found. T rash resolved rapidly in all cases after treatment with prednisone and cetirizine (10 mg/day). After resolution of rash, prednisone was discontinued, but rash recurred with the next bortezomib infusions despite continued treatment with cetirizine. To prevent recurrence of the rash, it was necessary to administer corticosteroids (10 mg prednisone) prophylactically before every administration of bortezomib. Two patients with bortezomib-associated rash were treated with dexamethasone together with bortezomib from 3rd cycle onwards due to minimal treatment response. In these patients, rash resolved and did not recur. Conclusions. VZV reactivation is common and serious consequence of bortezomib therapy. According to our experience, prophylaxis with acyclovir is very effective and should be considered for all patients treated by bortezomib. The minimal sufficient dose of acyclovir for prophylaxis of VZV reactivation needs to be established but in our group of patients dose of 400mg of acyclovir thrice daily was effective in 100% of cases. Rash is common toxicity seen in patients treated with bortezomib. Skin lesions are infiltrated by lymphocytes seem to be the most typical after bortezomib. According to our clinical experience, corticosteroids are useful for prevention and treatment of bortezomib-associated rash while maintenance treatment with antihistamines alone is not effective.

1228 SELECTED ELDERLY MYELOMA PATIENTS CAN BENEFIT FROM AUTOLOGOUS STEM CELL TRANSPLANTATION AND HAVE A SIMILAR CLINICAL OUTCOME AS YOUNGER PATIENTS: A SINGLE CENTRE STUDY
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High-dose chemotherapy with autologous stem cell transplantation (ASCT) is currently the standard treatment in myeloma patients below 65 years but transplant remains questionable in elderly patients. The aim of this study is to evaluate the feasibility and the efficacy of ASCT in elderly myeloma patients. We retrospectively reviewed the medical files of forty seven patients with stage II or III multiple myeloma treated with high doses melphalan followed by ASCT between 1995-2005. We compared the clinical outcome of patients older than 65 years patients with the outcome of the ASCT to younger ones. Progression-free survivals (PFS) and overall-survivals (OS) curves were compared using the Kaplan-Meier model on an intent-to-treat basis. Forty seven symptomatic myeloma patients treated with ASCT were followed: 10 patients were 65 years or more and 57 patients were younger. Median age at the time of ASCT was respectively 67 years (65-71) and 55 years (39-69). There were no significant differences in the distribution of pre-treatment characteristics: β2 microglobulinemia, chromosome 13 deletion, renal dysfunction, stages (two patients were diagnosed stage II and forty five at stage III), PS and number of comorbidities. Sixty eight percent of the patients had none or single comorbidity. There were no significant differences in median PFS between patients older than 65 years and younger (13 months versus 17 months, p=0.36) and in median OS, respectively 57 months versus 59 months (p=0.32). A trend to a better remission rate was observed in younger patients (p=0.09) but good partial remission rate was similar in both groups of patients. Transplant related mortality (TRM) was 0% and serious adverse events (SAE) are similar in both groups (54% vs 36%). Clinical outcome was similar in both groups of patients treated by ASCT. OS, TRM and SAE of elderly patients (65+) were improved compared to previous studies. These differences could be explained by the population of fit elderly patients with few adverse prognosis factors (β2 microglobulinemia, chromosome 13 deletion, renal dysfunction) in our study. Selected elderly patients with few comorbidities, can benefit from ASCT and have a similar clinical outcome as younger patients.

1229 PLASMA CELL IMMUNOPHENOTYPE CD56 POSITIVE AS GOOD PROGNOSIS MARKER
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Background. Malignant plasma cell could have a classical appearance, or it could be atypical at the optical microscopy analysis. This is known to be a B-cell without expression of lineage markers (CD19, CD20), with lack of CD45 expression and typical expression of CD38, CD138, and CD56. There were described aberrant coexpressions of CD10, CD28, c-kit, and CD138 in a few cases. Aims. We have analyzed our cases of multiple myeloma and plasma cell leukemia to correlate with the immunophenotype and microscopic appearance. Methods. We performed optical microscopy and immunophenotyping on peripheral blood cells and bone marrow aspirate, in 67 patients, with median age of 63 years. Results. We found 5 cases of typical immunophenotype CD56, CD138, and CD38 positive patients. Immunophenotyping by flowcytometry found in 70% of patients the expression of CD38, CD138 and CD34. In 15% we found lack for CD56 and in 20% lack for CD38. We found also that those patients with lack for CD34 had poor prognosis and the lack for CD38 didn’t change the prognosis. In a patient with plasma cell leukaemia positive expression of CD56 could be considered as good prognosis marker, despite of aberrant lack of CD38 expression on plasmablasts. Aberrant coexpression of CD20, CD138 or CD34 didn’t associate poor outcome. In 5% patients we found plasma cells in peripheral blood associated with poor prognosis and terminal phase of disease. In conclusion, we suggest that immunophenotyping in plasma cell leukemia and multiple myeloma is very important, and critical for the quickly diagnosis, too. We can find important prognostic markers, and we consider that lack of expression of CD56 could be the most important, associated with poor prognosis.

1230 MORPHOFUNCTIONAL STATUS OF LIVING PLATELETS AND THROMBOSIS RISK IN PATIENTS WITH CHRONIC RENAL FAILURE AT THE END STAGE OF HEMODIALYSIS
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Backgrounds. Heparin-induced thromboctopenia and thrombosis is a severe complication in patients on hemodialysis. To appreciate the character of cellular hemostasis disorders in patients with chronic renal fail-
Heparin is a potent anticoagulant used in acute venous or arterial thromboembolism. Unfortunately, individual differences can be seen in anticoagulant response of patients that are treated with heparin. Activated partial thromboplastin time (APTT) is the most widely used monitoring test. The therapeutic range for APTT can be different such as 1.5-2.5, 1.5-2, 1.5-3 times of the normal laboratory mean in the literature. APTT is better correlated with the risk of heparin induced thrombocytopenia or its antithrombotic effect. Different APTT reagents may have different responses to heparin. This may be the cause of the difference between the therapeutic ranges suggested in the literature. When we consider all of these data, making therapeutic range calibration for each APTT reagent corresponding to heparin levels 0.2 - 0.4 U/ml by prothamine sulphate titration or anti-Xa level of 0.3 - 0.7 U/ml may be an appropriate approach. Using anti-Xa assay is cheaper and easier than prothamine sulphate titration. The aim of this study is to determine the therapeutic APTT range by using anti-Xa assay and whether there is a difference between these old and new ranges. Besides, a poll is applied among doctors working in different wards of the hospital to understand that these two therapeutic ranges are different from the daily practice. APTT (STA CK Prest 5; Diagnostica Stago, France) and anti-Xa (STA-Rotachrom_ Heparin; Diagnostica Stago, Fransa) are studied in plasma samples of patients receiving heparin hospitalised in Internal Medicine and Neurology wards because of venous thromboembolism (VTE) or serebrovascular accident (SVA) between September 2002 and June 2003. The correlation between APTT and anti-Xa was analyzed by two variant correlation analysis and linear regression analysis. There was a very good correlation (r=0.78, p<0.001). The formulation of the correlation was as follow: APTT= 37 + (68.8xAntiXa). The therapeutic APTT range calculated by using 0.3 and 0.7 U/ml anti-Xa levels were 58-85 seconds. 1.5 - 2.5 times of these values were corresponding to 47.4 - 79 seconds. When a poll was made among 22 doctors treating VTE or SVA, it was seen that the therapeutic ranges used were different individually and from the values found in the study.

**1232**

Determination of the Relationship between Plasma Heparin Level and APTT and Ascertainment of the APTT Range to be Aimed During Treatment of Venous Thromboembolism by Heparin

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Heparin is a potent anticoagulant used in acute venous or arterial thromboembolism. Unfortunately, individual differences can be seen in anticoagulant response of patients that are treated with heparin. Activated partial thromboplastin time (APTT) is the most widely used monitoring test. The therapeutic range for APTT can be different such as 1.5-2.5, 1.5-2, 1.5-3 times of the normal laboratory mean in the literature. APTT is better correlated with the risk of heparin induced thrombocytopenia or its antithrombotic effect. Different APTT reagents may have different responses to heparin. This may be the cause of the difference between the therapeutic ranges suggested in the literature. When we consider all of these data, making therapeutic range calibration for each APTT reagent corresponding to heparin levels 0.2 - 0.4 U/ml by prothamine sulphate titration or anti-Xa level of 0.3 - 0.7 U/ml may be an appropriate approach. Using anti-Xa assay is cheaper and easier than prothamine sulphate titration. The aim of this study is to determine the therapeutic APTT range by using anti-Xa assay and whether there is a difference between these old and new ranges. Besides, a poll is applied among doctors working in different wards of the hospital to understand that these two therapeutic ranges are different from the daily practice. APTT (STA CK Prest 5; Diagnostica Stago, France) and anti-Xa (STA-Rotachrom_ Heparin; Diagnostica Stago, Fransa) are studied in plasma samples of patients receiving heparin hospitalised in Internal Medicine and Neurology wards because of venous thromboembolism (VTE) or serebrovascular accident (SVA) between September 2002 and June 2003. The correlation between APTT and anti-Xa was analyzed by two variant correlation analysis and linear regression analysis. There was a very good correlation (r=0.78, p<0.001). The formulation of the correlation was as follow: APTT= 37 + (68.8xAntiXa). The therapeutic APTT range calculated by using 0.3 and 0.7 U/ml anti-Xa levels were 58-85 seconds. 1.5 - 2.5 times of these values were corresponding to 47.4 - 79 seconds. When a poll was made among 22 doctors treating VTE or SVA, it was seen that the therapeutic ranges used were different individually and from the values found in the study.
response to ADP than the control group. The patient group of patients with VTE (8/17 vs. 1/59, p=0.055) has a significantly higher ratio of patients with low response to collagen than the control group. The whole patient group (12/46 vs. 2/59, p=0.001), the group of patients with VTE (5/17 vs. 2/59, p=0.005), and the group of patients with RFL (5/28 vs. 2/59, p=0.058) have a significantly higher ratio of patients with low response to epinephrine than the control group. Sticky platelet syndrome-like abnormality was detected in four cases (8.3%) of the patient group (one case with type I-like in RFL group, one case with type III-like in ATE group, two cases with type III-like in VTE group). Conclusions. Although we need data which will be obtained from many more cases for a certain evaluation, we suggest that in approaching the patients with thrombosis/RFL, platelet functions screening should be applied according the results of this preliminary report.

1234 PULMONARY EMBOLISM: EPIDEMIOLOGICAL CHARACTERISTICS ACQUIRED AND INHERENT RISK FACTORS

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Backgrounds. Pulmonary embolism (PE) is the most serious form of venous thromboembolism. Aims. To analyze the epidemiological characteristics and search for risk factors among cases of PE occurred in our area (North-Western Greece). Materials and Methods. The study group consists from 123 adults patients (mean age= 62, SD =14,8 years) presented in the emergencies with high PE probability. All patients treated in the Pneumonology Clinic during the last four years. Four patients with definite diagnosis of severe PE, with unstable cardiovascular function that treated on the Intensive Care Unit of our Hospital, are not included. The diagnosis of PE confirmed on 74 patients (64/123, 60%), which classified as idiopathic PE (IPE), 36 cases or as secondary PE (SPE), 38 cases, according to the co-existence of cancer, lymphoma, anti-phospholipid syndrome, recent trauma or operation and/or prolonged immobilization. Among the 49 patients that we did not confirm a diagnosis of PE (non-PE) only three were found with a condition that could accompany PE. During the follow-up one death occurred that attributed to secondary PE and two patients developed recurrence. Results. Although the women and the older presented more frequently as cases of SPE neither the sex nor the age was statistically different between the cases of IPE, SPE or non-PE. Fibrinogen and D-Dimers levels are significantly higher among IPE patients. Promoter MTHFR C677T (C->T), 64 (min 30-max 80 y) - developed a VTE: 11 deep vein thrombosis, 3 pulmonary embolism (PE), 10 venous thromboembolism (VTE) (PE and DVT), 1 stroke and 3 patients died. We compared several rapid methods, and have chosen VIDAS D-Dimer by bioMerieux. The goals of this study were: 1. Following up patients with normal DD levels, who were excluded from further testing, in order to test the reliability of our method of choice. 2. Evaluating the cost-effectiveness of the use of this test. 3. Evaluating whether the test is employed according to the original indications given to the medical staff. In the first phase of the evaluation we compared the results of DD tests of a random group of 60 patients suspected of VTE to the conservative way of diagnosis of VTE that is practiced in our institution. Patients were followed for 6 months to rule out recurrent events or misdiagnosis. 57.0% of the patients showed normal DD levels, and none of these patients was diagnosed with VTE during the follow-up. Of the patients with elevated DD levels, only 27% were independently diagnosed with VTE. These results led us to instruct the medical staff to perform the DD test only on patients accepted to the emergency department with no underlying medical problem, and who have low to moderate probability for VTE. The test showed a good correlation in 72 patients with normal DD levels, and none of them was misdiagnosed with VTE.

Venus thromboembolism (VTE) is a common clinical entity in most emergency departments and often requires extensive diagnostic analysis. DD test is recognized as a valuable tool for screening patients suspected of VTE, allowing to ascertain the absence of thrombosis. We compared several rapid methods, and have chosen VIDAS D-Dimer by bioMerieux. The goals of this study were: 1. Following up patients with normal DD levels, who were excluded from further testing, in order to test the reliability of our method of choice. 2. Evaluating the cost-effectiveness of the use of this test. 3. Evaluating whether the test is employed according to the original indications given to the medical staff. In the first phase of the evaluation we compared the results of DD tests of a random group of 60 patients suspected of VTE to the conservative way of diagnosis of VTE that is practiced in our institution. Patients were followed for 6 months to rule out recurrent events or misdiagnosis. 57.0% of the patients showed normal DD levels, and none of these patients was diagnosed with VTE during the follow-up. Of the patients with elevated DD levels, only 27% were independently diagnosed with VTE. These results led us to instruct the medical staff to perform the DD test only on patients accepted to the emergency department with no underlying medical problem, and who have low to moderate probability for VTE. The test showed a good correlation in 72 patients with normal DD levels, and none of them was misdiagnosed with VTE.

1235 THE USE OF D-DIMER TEST IN DIAGNOSIS OF VTE IN EMERGENCY ROOM SETTING: EXPERIENCE OF CARMEL MEDICAL CENTER

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Venous thromboembolism (VTE) is a common clinical entity in most emergency departments and often requires extensive diagnostic analysis. DD test is recognized as a valuable tool for screening patients suspected of VTE, allowing to ascertain the absence of thrombosis. We compared several rapid methods, and have chosen VIDAS D-Dimer by bioMerieux. The goals of this study were: 1. Following up patients with normal DD levels, who were excluded from further testing, in order to test the reliability of our method of choice. 2. Evaluating the cost-effectiveness of the use of this test. 3. Evaluating whether the test is employed according to the original indications given to the medical staff. In the first phase of the evaluation we compared the results of DD tests of a random group of 60 patients suspected of VTE to the conservative way of diagnosis of VTE that is practiced in our institution. Patients were followed for 6 months to rule out recurrent events or misdiagnosis. 57.0% of the patients showed normal DD levels, and none of these patients was diagnosed with VTE during the follow-up. Of the patients with elevated DD levels, only 27% were independently diagnosed with VTE. These results led us to instruct the medical staff to perform the DD test only on patients accepted to the emergency department with no underlying medical problem, and who have low to moderate probability for VTE. The test showed a good correlation in 72 patients with normal DD levels, and none of them was misdiagnosed with VTE.

1236 VENOUS THROMBOEMBOLISM (VTE) IN HAEMATOLOGICAL MALIGNANCIES: A SINGLE CENTRE EXPERIENCE

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Backgrounds. The overall risk of VTE is increased 7 fold in cancer patients. Within cancer population the highest risk of VTE is recorded in pts with hematological disorders (OR ratio 2.8), lung and gastrointestinal cancer, respectively (Blom, 2005). Aims and Methods. The aim of this retrospective study was to evaluate: a) the overall incidence of VTE in our patients with haematological malignancies, b) the incidence of a thrombophilic status in these patients. Thrombophilic screening consists of Factor V Leiden and Factor II polymorphism, PC, PS and ATIII activity, LAC and ACA Ig M and Ig G assays. From January 2004 to December 2005, 259 consecutive patients with haematological disease (HD), 71 Acute Leukemias (AL), 34 Hodgkin Disease (HD), 60 Non Hodgkin’s Lymphomas (NHL), 64 Myelodisplasia (MDS), 30 Multiple Myeloma (MM) - entered in this study. Patients with myeloproliferative disorders were not included since in these cases it is well known VTE represents a frequent feature of disease. 3.Results. Of the 259 patients, 20 (7.7%)- 5 males, 12 females, median age 64 y (min 25-max 80 y)- developed a VTE: 11 deep vein thrombosis, 3 inferior vena cava, 6 upper right arm. VTE occurs in 6 (8.4%) AL, 4 (11.2%) HD, 7 (12%) NHL, 1 (1.5%) MDS, 2 (6.6%) MM. 15 patients had concomitant diseases: cardiac (8), renal (1), solid cancer (1), metabolic disorders (5); furthermore 6 of these had central venous catheter, 5 were receiving chemotherapy, 3 had haematological disease status at time of VTE; 15 cases had active disease (5 at onset, 11 in relapse or progression) while 5 were in complete remission (CR). Thrombophilic screening was available in 16 patients. Abnormal
tests were detected in only 4 (25%) cases. AICA IgG high titer (1), reduced PC activity (1), hyperhomocysteinemia (2). In all cases VTE treatment has been successfully done with subcutaneously LMWH for 4 months at least. To date 18 of 20 patients are alive: 11 in haematological CR, 7 in stable disease, 2 patients died because of progressive disease. 4. Conclusions. In our series, if small, 75% of patients who developed VTE had an active phase of haematological disease, VTE incidence was higher in lymphomas than in other malignancies. Where a normal thrombophilic tests were detected in only 25% of cases. The endovascular vascular damage induced by central venous catheter plus chemotherapy proved to be determinant in the development of upper right arm VTE.

1237
IS OSTEONECROSIS ASSOCIATED WITH MUTATIONS OF THE METHYLENETETRAHYDROFOLATE REDUCTASE GENE?
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Backgrounds. Mutations of the methylenetetrahydrofolate reductase (MTHFR) gene interface with homocysteine metabolism, and hyperhomocysteinemia is considered a risk factor for thromboembolic complications. Intravascular coagulation is considered as one of the major pathogenic pathways leading to ischemic bone death (osteonecrosis) and the role of thrombophilic gene mutations is increasingly being recognized. Aims. The purpose of our study is to investigate the presence of MTHFR mutations in patients with osteonecrosis (ON) in an effort to clarify the complex pathogenesis of the disease. Methods. We evaluated a patient group of 48 consecutive adults with ON and a control group of 48 healthy blood donors. All controls were matched for race, age, and gender to the patients and had no history of cardiovascular disease or thromboembolic events. Genetic analysis of the MTHFR C677T and A1298C polymorphisms was carried out by allele-specific polymerase chain reaction. Results. Homozygosity for the MTHFR C677T mutation was present in 6.3% (3/48) of ON patients compared to 8.3% (4/48) of controls. The difference was not statistically significant, with an odds ratio of 0.79 (95% confidence interval 0.2 to 3.5). Homozygosity for the MTHFR A1298C mutation was present in 12.5% (6/48) of ON patients compared to 10.4% (5/48) of controls. The difference was again not statistically significant, with an odds ratio of 1.2 (95% confidence interval 0.3 to 4.3). Conclusions. Although hyperhomocysteinemia is considered a thrombophilic factor, the potential pathogenetic role of the C677T and A1298C MTHFR mutations in thromboembolic disease remains controversial. In the current report, we detected no differences in the prevalence of these mutations in patients with ON compared to controls. Intravascular coagulation in patients with ON may be mediated by other genetically determined factors.

1238
DERMATAN SULPHATE THERAPY FOR HEPARIN-INDUCED THROMBOCYTOPENIA
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Heparin-induced thrombocytopenia (HIT) is an acquired hypercoagulable state. As soon as HIT is suspected, heparin should be discontinued and non-heparin anticoagulant should be started. Lepirudin, Argatroban and Danaparoid are among the alternative anticoagulants more investigated in HIT. Dermatan sulphate (DS) is a safe, effective and inexpensive therapeutic option for HIT though clinical experience with its use is limited. We report our clinical experience on DS therapy in patients with HIT. HIT with thrombotic syndrome was clinically suspected in 3 patients according with the Warkentin’s criteria (NEJM 2001; 344:1256-1292). Laboratory confirmation of HIT was obtained by an ELISA test for anti-FP4-heparin antibodies in two patients. Venous thromboembolism was confirmed by imaging tests. DS (Mediolanum Farmaceutici, Milan, Italy) was administered by intravenous continuous infusion in 6 patients at dose of 0.6 mg/kg/h, the infusion was regulated monitoring APTT every 6-12 hours and targeting APTT of 1.5-2 times the normal value. Platelet count was close monitored. Warfarin was started in two cases when platelet count raised over 100 x 109/L, in one case before DS infusion. DS was stopped when INR was in therapeutic range for two consecutive values. The cost by vial of DS is 0.9175 € in Italy. Patients included were two females (59 and 81 years old) and one male (65 years old). In one case HIT developed during administration of unfractionated heparin for lower limbs deep venous thrombosis (DVT) and the course was complicated by sinus thrombosis, in two cases after antithrombotic prophylaxis with low-molecular-weight heparin: one, after treatment of lower limbs DVT and pulmonary embolism, the other, with entero-vascular fistula, suffered of upper limbs deep vein catheter-related thrombosis and pulmonary embolism. In 2 patients DS was started with platelet count of 49-129 x 109/L and platelet count raised over 150 x 109/L after 3-5 days, DS infusion was continued for 11 and 13 days; in patient, taking Warfarin, DS was stopped with platelet count of 109 x 109/L and DS was stopped with platelet count of 62 x 109/L. No bleeding complications or adverse events were observed, clinical improvement was observed in all patients. Patient with entero-vascular fistula, few weeks later HIT, was operated and antithrombotic prophylaxis with DS offered an uneventful outcome. DS total therapy cost for two patients, before warfarin was in therapeutic range, was respectively of 26.7 euro and 56 euro. DS appears an effective and safe therapeutic option for patients with HIT. Our experience, together with other reports in which patients with HIT were treated successfully, encourages using this alternative anticoagulant drug in HIT therapy and as postoperative antithrombotic prophylaxis in patients with recent history of HIT. DS shows also a favourable profile cost-benefit.

1239
THROMBOMODULIN, SEPCR AND DI-DIMERS AS PLASMA MARKERS OF ENDOThelial DYSFUNCTION IN WOMEN WITH A HISTORY OF RECURRENT PREGNANCY LOSS
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Recent pregnancy loss can be associated with endothelial disturbance (whether activation, dysfunction or damage). Thrombomodulin (TM) and the endothelial protein C receptor (EPCR) are glycoprotein receptors expressed mainly on the endothelial surface of blood vessels and also in the placenta. They both play a key physiological role in the protein C anticoagulant pathway. Defects in these proteins might play an important role in the pathogenesis of fetal loss. So we decided to study Di-Dimers, TM and SEPCR as early markers for the beginning and prognosis of recurrent pregnancy loss. We studied 102 women with unexplained fetal loss and 44 women as control group. We used an immunologic assay (Dade Behring) for calculating Di-Dimers and ELISA (Asserochrom ASTAGO) for TM and SEPCR measurement. The levels of DI-Di were 189,28±13,7 µg/L in patients group and 172,02 µg/L in control group (p=0,46). TM levels were 9,78±2,4 ng/mL in patients group and 8,3±2,7 ng/mL in controls (p=0,017). There was not statistical difference in SEPCR measurement (187,2 ng/mL versus 160,7 ng/mL in control p=0,22). TM may serve as a clinically meaningful endothelial injury marker in women with a history of recurrent pregnancy loss. Further investigation is needed to see the significance of other factor as Di-Di and SEPCR.

1240
THE ABO BLOOD GROUPS, FVIII, FIX, VWF LEVELS AND LEIDEN IN PATIENTS WITH THROMBOSIS IN GREECE.
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Increased levels of FVIII, FIX, VWF in plasma as the presence of FV leiden represent an important risk factor for venous thromboembolic disease. There is also a relationship between these factors and ABO Blood groups. We investigated the influence of the ABO blood group in Greek patients with thrombosis and the association with raised plasma levels of the above coagulation factors. 159 patients of median age 48,5 ± 5,4 y (97 females and 62 males) were included in our study. They have visiting our hospital for first thrombotic event when younger than 60 years old. Patients with malignancies or with history of liver failure or nephrotic syndrome were excluded. As controls 60 (36 F and 24 M) apparently healthy individuals were recruited. We measured the plasma levels of
FVIII, FIX, vWF, and also we determined the ABO type blood and the presence of FV Leiden. There is a high prevalence of thrombosis, in the non-O blood group (102/57), especially in patients of A or AB type blood. The plasma levels of FVIII, IX and vWF were 112.6%, 82.4%, and 136.5% respectively in the patient groups and 92.5%, 89.2% and 99.5% in control group. There were 14 carriers of FV Leiden (8.7%) in patients group and 5 carriers in healthy controls (3.5%). We conclude that: I. there is an association between non-O blood type and thrombosis in Greek patients; 2. FVIII, vWF levels and FV Leiden were significant higher in individuals with non-O blood type.

**1241**

**THE CONTRIBUTION OF PROTHROMBIN AND MTHFR MUTATIONS TO VENOUS AND ARTERIAL THROMBOEMBOLISM IN CARRIERS OF FACTOR V LEIDEN**

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**Backgrounds.** Given the multifactorial aspect of thrombophilia, the identification of combined genetic factors in patients with thrombotic episodes is important to a more accurate risk assessment. These patients are routinely screened for Factor V Leiden (G1691A), prothrombin G20210A, and MTHFR C677T gene mutations at the American University of Beirut Medical Center (AUBMC). It is not known, however, if the presence of prothrombin or MTHFR gene mutations increases the risk in carriers of Factor V Leiden mutation whether they had venous or arterial thrombosis. **Aims.** We assessed the contribution of prothrombin and MTHFR to the thrombotic risk in patients with venous or arterial thromboembolism. **Methods.** The population in our study consisted of a group of patients presenting with thrombosis over a period of 18 months. Patients were screened for the three most common thrombophilia genetic mutations namely Factor V Leiden (G1691A), prothrombin G20210A and MTHFR C677T. A group of healthy controls was also included in the analysis. The DNA of patients and controls was extracted using the FEL-FREEZ extraction kit (FEL-FREEZ, DYNAL, USA) and stored at -80°C for later use. Simultaneous testing for all three mutations was done using the Reverse Hybridization StripAssay (Vienna Lab). Extraction, PCR amplification, and Hybridization steps were all followed upon the recommendation of the manufacturer.

<table>
<thead>
<tr>
<th>Arterial thrombosis</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebrovascular accident</td>
<td>25</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>9</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>7</td>
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</table>

**Results.** The sample included 41 patients with arterial thrombosis, 99 patients with venous thrombosis, and 125 healthy controls. The average age of the three groups was 42.8±19.4, 39.6±17.3, and 35.4±18.6 years respectively. Table 1 shows the clinical presentation of patients with thrombosis. Patients with venous thrombosis were 7.1 times more likely to have Factor V mutation (heterozygous or homozygous) as compared to the control group. On the other hand, neither prothrombin nor MTHFR mutation was significantly associated with venous thrombosis. A logistic regression was conducted to test whether prothrombin and MTHFR mutations increase the risk of venous thrombosis among subjects with Factor V mutation. None of the two factors was found to be significantly associated with venous thrombosis after controlling for Factor V. Patients with arterial thrombosis were 2.5 and 4.4 times more likely to have Factor V (p=0.04) and prothrombin (p=0.04) mutations compared to the control group. There was no significant association between MTHFR mutation and arterial thrombosis. **Conclusion.** We investigated Factor (F) FIX and FIXa in human semen. We assessed the contribution of prothrombin and/or MTHFR mutations to venous or arterial thromboembolism in carriers of Factor V Leiden carriers. However, in patients with arterial thrombosis, the risk is increased in Factor V Leiden carriers with prothrombin mutation. These results might have an influence on the risk assessment and management of patients with arterial thrombosis. However, no final conclusions can be made from our results because of the small sample size. It would also be rational to conduct similar studies with stratification of patients into subgroups based on the definite site of thrombosis.

**1242**

**ARE SEMINAL FACTORS IX AND XA INVOLVED IN THE SEMINAL COAGULA GENETIC FORMATION?**

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**Backgrounds.** In spite of evidence demonstrating the importance of the seminal coagulation and liquefaction process in terms of global fertility and that the seminal coagulum is composed of fibrin-like material, it has rarely been studied from the conventional haemostatic factors perspective. **Aim:** To investigate Factor (F)FIX and FIXa in human semen. **Materials and Methods.** Using a one stage factor assay based on PT/PTT and spectrozyme FIXa assay FIX and FIXa were studied in a total of 119 semen specimens obtained from sub-fertile, normally fertile, fertile sperm donors and vasectomy subjects. Results. Both FIX and FIXa were quantifiable in human semen. There was a wide individual variation in FIX and FIXa levels within groups. Despite the group size, statistically significant associations with fertility-related parameters were infrequent. There was a positive correlation between FV and its activation product, FIXa (r=0.56; p=0.01). FIXa elevation in the high sperm count group was significant (p<0.05) and days of abstention correlated with FIXa levels (n=63; r=0.3; p<0.05). **Conclusion.** The key finding of this study is that both FIX and FIXa are present in concentrations not dissimilar to plasma levels and apparently functional, as the activated form is also present.

**1243**

**PREVALENCE OF FACTOR V G1691A, FACTOR II G20210A AND MTHFR C677T POLYMORPHISMS IN PATIENTS WITH DEEP VENOUS THROMBOSIS**

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**Backgrounds.** The risk of thrombosis may occur through the interaction of both genetic and acquired factors. The predisposition towards thrombosis increases with the number of risk factors present in the patient. **Aim:** The aim of this study was to evaluate the independent and combined effect of Factor V G1691A (FV-Leiden, FVL), Factor II G20210A (PT II) and methylene-tetrahydrofolate reductase C677T (MTHFR) polymorphisms on the incidence of deep venous thrombosis (DVT). **Patients and Methods.** We enrolled 128 patients with first episode of DVT (65 males, 63 females) and 186 healthy individuals (83 males, 103 females). FV, FII and MTHFR genotypes were analyzed using PCR amplification. We calculated odds ratio (OR) with 95% confidence intervals (CI), adjusted for gender and age by means of multiple logistic regression. **Results.** The prevalence of the heterozygote and homozygous variants for FVL (25.0% vs 6.5%, p<0.001) and PTH (10.2% vs 3.2%, p=0.01) were higher among DVT patients compared with controls. However, the presence of the T/T genotype for MTHFR was not different between the two groups (4.4% in patients vs 8.1% in control group, p=0.64). In order to identify independent and combined effect of the above mutations on the incidence of DVT, we divided the entire cohort into seven groups according to the presence of none, one or two mutations. The combination of the three mutations was not detected. The group without any mutation was used as reference group. Both FVL and PTH significantly increased the risk for DVT compared to the reference group (FVL: OR=4.0, 95%
The amorphous gelatinous substance was identified as acid mucopolysaccharide on alcian blue staining at pH 2.5. On a bone marrow biopsy, granulopoiesis was reduced and in the intratrabecular space eosinophilic extracellular material and fat cell atrophy, consistent with gelatinous bone marrow transformation was found. Summary/Conclusions. GMT is a rare disorder that is associated with various underlying diseases, the most frequent being anorexia nervosa and the acquired immunodeficiency syndrome (AIDS). Although frequently associated with weight loss, the bone marrow changes have not been associated with any specific deficiency state and their pathogenesis has not been fully elucidated. GMT may act as an indicator of severe illness in a patient but is not indicative of a particular disease. It is an uncommon cause of cytopenia and should be considered in the setting of malnutrition.

Figure 1. Bone marrow aspirate stained with May-Grünwald Giemsa.
or to therapy and also an international protocol for managing patients at risk for reactivation of hepatitis B virus in high prevalent areas such as Iran should be carried out.

1247
A 3 YEAR SURVEY OF STRAINS IDENTIFIED IN BLOOD CULTURES IN A CLINICAL HEMATOLOGY UNIT
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Febrile neutropenic cancer patients are at the risk for development of serious infections, morbidity and mortality. Among these infections, bacteremia had a place of choice and is associated with a strong mortality. The microbiological documentation is not always present, the antibiotic therapy remain probabilist inspired of the ecology of the service. The aim of this study is to analyze the bacteriological profile of bacteremia in a clinical haematology unit in order to guide better the antibiotic therapy of first intention. All the microorganisms(n=138) collected over 3 years(January 2003 to December 2005), from blood cultures of hospitalized patients in the clinical haemotology unit were studied. Antimicrobial susceptibility testing has been carried out by disk diffusion method in accordance with the French Society of Microbiology. All susceptibility data were stored in a laboratory data base using Whonet software. Duplicate isolates defined as the same bacterial species for the same patient with the same profile of susceptibility were excluded. Gram positive cocci rate(GPC) was 60,1% and Gram negative bacilli(GNB) 39,9%. Evolution in time showed an equal rate between GPC and GNB in 2005(51,4% versus 48,6%), however a decrease in GPC rate isolated in bacteremia was observed in 2004 (63,6%) and 2005 (62,3%).The most frequently identified species were coagulase-negative staphylococci(CNS): 29,3% and 50% in 2003 and 50,8% in 2005, no VISA (vancomycin intermediate S. aureus) was detected during the study period. P. aeruginosa resistance was 33%, 4,3%, 30,8%, 40% respectively for ceftazidime, imipenem and amikacin. Concerning K.pneumoniae, 86,7% of strains were resistant to ceftazidime, 46,7% to ciprofloxacin and 85,7% to amikacin. The frequencies of resistance to ceftazidime, ciprofloxacin and amikacin of E.coli were respectively 50%, 33,3% and 41,7%.Imipenem and colistin were the most active antibiotics against K. pneumoniae and E. coli (resistance rate= 0%). Bacteremia were mainly caused by coagulase-negative staphylococci during the three study years. Multiresistance of germs isolated is worrying limiting the therapeutic choice. Ongoing cooperation between haematologists and microbiologists is important to detect trends in epidemiology which can be used to design empirical antibiotic regimens and guide infection control policies.

1248
BACTERIAL FLORA AND ANTIBIOTIC RESISTANCE IN A CLINICAL HEMATOLOGY UNIT
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Infections are among the most serious complications in neutropenic patients and are associated with an increased morbidity and mortality. Ongoing surveillance of infection in neutropenic patients is essential to detect changes in epidemiology and to guide better empirical antibiotic regimens and infection control policies. The aim of this study is to analyze the bacterial flora and the antibiotic resistance of isolates in a clinical haematology unit during three years period. From 1 January 2003 to 31 December 2005, 437 strains were isolated from different specimens. Antimicrobial susceptibility testing has been carried out by disk diffusion method as referred to the French Society of Microbiology. All susceptibility data were stored in a laboratory data base using Whonet software. Duplicate isolates defined as the same bacterial species for the same patient with the same profile of susceptibility were excluded. Gram negative bacilli(GNB) rate was 45,3% and 21,3% and 26,3% and 23,1%. Concerning K. pneumoniae, 84, 8% of strains were resistant to cefazidime and were producing extended spectrum β-lactamase (BLSE). The evolution in time showed an increase in rate of K. pneumoniae BLSE: 57, 1% in 2003 versus 95, 5% in 2005. All strains of K. pneumoniae isolated remained sensitive to imipenem and colistin. Concerning P. aeruginosa, 50% of strains were resistant to ceftazidime, 50% to imipenem, 51, 6% to amikacin. An increase of the imipenem resistance in P. aeruginosa was observed from 2003 to 2005(28, 6% in 2003 versus 45, 5% in 2005). The incidence of antimicrobial resistance has markedly increased during 2005, especially for the ceftazidime in K. pneumoniae (95, 5% in 2005 versus 57, 1% in 2003) and the imipenem in P. aeruginosa (45, 5% in 2005 versus 26, 8% in 2003). After this study, a restriction of the use of ceftazidime which was utilized in the first antibiotic therapy was instaurated in the unit. The ongoing surveillance of antimicrobial resistance in the hematology unit should be present be helpful in formulation of effective guidelines for therapy.

1249
PROPHYLAXIS OF INVASIVE FUNGAL INFECTIONS (IFI) IN ACUTE NON LYMPHOID LEUKEMIA (ANLL): EFFICACY OF AMPHOTERICINE B LIPID COMPLEX (L-AMB) SINGLE LARGE DOSE DURING INDUCTION
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Up to now, the optimal prophylactic regimen to prevent IFI in ANLL is not yet been identified. The L-AMB has been used in patients refractory or intolerant to other antifungal drugs, although long time is required for a relevant oversirve infection and this event occurred ANLL therapy. Therapeutic plan. The efficacy of L-AMB seems to be related both to improved tissue penetration and to sustained bioactivity of drug levels in lung, brain, kidney, liver, spleen (Anaissie et al. 2004). On the basis of this issue ,we have planned a pilot study for IFI prophylaxis in de novo ANLL to test the efficacy and safety of a single large dose of L-AMB (15 mg/kg) during post induction neutropenia. Primary endpoint: to evaluate the incidence of fungal infection according to International Consensus (Ascioglu et al. 2002) and during up to four weeks after prophylaxis. Patients: the study started in may 2005 and it is still open ,as of January 2006. 52 consecutive adult ANLL (4 APL patients - 14 M, 7 F, median age 57 yrs (range 39-75) - are enrolled. Intensive induction chemotherapy included standard/high dose cytosine-arabinoside + antaclycines + etoposide and retinoic acid + antaclycines in APL. Methods. Inclusion criteria were: 1) neutropenia (PMN <0.5x10^9/L) longer than ten days; 2)surveillance cultures, mannano and galattomannano antigens negative; 3) no fever and/or clinical signs of infection. On the day after the end of induction, patients received a single dose of L-AMB (Ambisome, Gilead®) at 15 mg/kg i.v. A second dose was repeated 15 days later in those cases persistently neutropenic and who met inclusion criteria. L-AMB PK profile was tested in 15 patients at the following times: 0.1,42,4 hours, 7th and 14th day from drug administration. Results. 15/21 patients(72%) achieved complete haematological remission,1 partial remission, 1 was resistant and 4 died during induction aplasia .Overall median duration of neutropenia was 22 days(range 16-42). The median dosage of L-AMB was 900 mg/dose (range 750-1200 mg); in six cases a second single dose was administered. Of 21 patient entered in this study,17 (81%) did not developed fungal infection , 3 cases had IFI (1 Candida spp sepsis and 2 Aspergillosis, respectively) and 1 died early. As L-AMB related toxicities, only 2 patients had CTC grade II allergy, promptly recovered by i.v. steroids. The median L-AMB PK results are, to date, available in 8 patients: 0 h <0,15, 1 h 8,92±4,25; 4h, 51,26 ±26,7; 24h, 3,92±11,77; 7th, d 1,39±1,97; 14th, d, 0,27±0,092; (<lower standard rate 0.45 mg/l ± standard deviation). Comments: The reported results, if obtained in a single center, seems that L-AMB single large dose is an effective and safe approach in IFI prophylaxis. In fact 81% of treated patients did not develop fungal infections and side effects were not significant. Furthermore the preliminary results of PK profile demonstrated an early high plasma levels of the drug which slowly cleared until 14th day.
RECURRENT DISSEMINATED SKIN LESIONS DUE TO METARRHIZIUM ANISOPLIAE IN AN ADULT PATIENT WITH ACUTE MYELOGENOUS LEUKAEMIA

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Case report. A 62 year old male was diagnosed in April 2005 with acute myelogenous leukaemia. On day +42 after two cycles of induction chemotherapy, disseminated skin papules with central ulceration appeared involving the face, trunk, and limbs. A skin biopsy yielded dermoeosdermic necrosis and fibrin thrombi. Not cultures were obtained. The patient was receiving antibiotics and caspofungin for persistent neutrogenic fever. He became afibrile and recovered from his neutropenia four days later. Caspofungin and antibiotics were withheld. The skin lesions gradually improved. Two more cycles of chemotherapy were administered and a new two lesions appeared after the fourth cycle. They gradually resolved. During September and October 2005, while awaiting an autologous stem cell transplantation (SCT), disseminated skin lesions reappeared. A new skin biopsy was performed and wasinitially interpreted as an acute inflammatory dermal lesion with a mixed neutrophilic and histiocytic infiltrate. During this time most of the biopsies underwent spontaneous resolution, but new papules appeared. On November 2005 he was admitted to undergo an autologous SCT. He had then 4 skin lesions in resolution. Deeper sections of the second skin biopsy revealed a nidi of fungi in the dermis with broad hyphae. A new skin biopsy showed similar features. Biopsy cultures and a galactomannan test were negative. A chest CT scan was normal. Tissue samples were sent to the Mycology Reference Laboratory of Spanish National Center for Microbiology. Specimens were analysed using a panfungal PCR-based assay designed to amplify the internal transcribed spacer regions 1 and 2 from fungal rRNA gene complex. Subsequent sequencing of amplified fragments and comparison with sequences of other fungal species included in databases led to know that the DNA amplified from tissues belonged to the fungal species Metarrhizium anisopliae. During transplantation no new lesions appeared. On day +9 he was started on liposomal amphotericin B for neutrophic fever but it was discontinued after one dose because of a severe infusion reaction. He did not receive any further antifungal therapy and the skin lesions resolved. In December 2005, he showed 2 new skin papules. A biopsy was performed and cultures and PCR for fungal DNA were not positive. Voriconazole was started and the lesions disappeared. Treatment was discontinued after a month. No further skin lesions appeared. Discussion: We report the first case of a probable disseminated infection caused by M. anisopliae in an adult patient. The organism was not isolated from any of the four samples and was identified by PCR and sequencing. This case exemplifies the clinical usefulness of molecular methods to diagnose mycosis due to emerging pathogens. Metarrhizium anisopliae is a common insect pathogen and occasionally causes infection in animals and humans. To date, there are only 3 reported cases of disease in humans: two of keratitis, two of sinustis, and one of a disseminated invasive infection in an immunocompromised child. There is no standard treatment. Susceptibility testing suggests that M. anisopliae may be resistant to amphotericin B, 5-Flucitosine, and fluconazole. Itraconazole and Voriconazole could be more effective agents.

AUDIT OF THE USE OF ANTIFUNGALS AND THE ACTUAL RATES OF FUNGAL INFECTION ACCORDING TO EORTC/MSG CRITERIA IN PATIENTS UNDERGOING HIGH DOSE CHEMOTHERAPY FOR AML AND/OR ALLOGENEIC TRANSPLANTATION

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Background. The high morbidity and mortality of fungal infections in neutropenic patients has led to prophylactic and empirical drug regimens. Antifungal drug use and actual rates of invasive fungal infection (IFI) may differ considerably. Aims. To audit the use of antifungal drugs in patients with AML and/or allograft transplant recipients admitted to our hospital from 01/01 to 31/12/2004 and to apply the EORTC/MSG criteria (1) for IFI. Methods. The medical notes were retrospectively reviewed for: conversion from prophylactic to empirical treatment; time to neutrophil recovery; duration of hospitalisation and mortality. Primary prophylaxis was with fluconazole 400mg od, while empirical therapy (ambisome) was prescribed for persistent neutropenic fever despite 72-96 hours of antimicrobials. Those patients, who did not meet EORTC/MSG possible criteria, were termed unlikely. Galactomannan testing was not done routinely in our hospital. Results. 54 patients (out of 77 eligible) were assessable, providing 137 episodes: 75% underwent intensive chemotherapy for AML/MDS, 18% allogeneic transplantation and 7% supportive treatment for neutropenic sepsis on the background of AML. 114/137 episodes (85%) received primary prophylaxis - oral fluconazole (78%); 21/137 (15%) secondary prophylaxis - oral voriconazole. 2% did not receive prophylaxis. The conversion rate from prophylaxis to empirical therapy was 25% (85/343), mainly due to persistent neutropenic fever. These 35 episodes concerned 27 of the 54 patients (50%). The EORTC/MSG infection rates are shown in Table 1.

Table 1.

<table>
<thead>
<tr>
<th>EORTC-defined infection</th>
<th>Number of episodes that were started on empirical treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proven</td>
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</tr>
<tr>
<td>Probable</td>
<td>4/35 (11%)</td>
</tr>
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<td>Possible</td>
<td>18/35 (51%)</td>
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</tbody>
</table>

Of these 35 episodes only 4 (11%) were probable IFI - all had HRCT evidence of IFI with negative bacterial cultures. Another 22 episodes involved HCRTs: 11 had normal results, and 11 had non-specific changes. Thus, HRCT was the main diagnostic test in the small total number of probable infections. Patients in 25 of the 35 episodes on treatment (71%) were hospitalised for 2-25 days (range 29-60, median 34). Their EORTC/MSG IFI score was: 13 possible, 5 probable, 6 unlikely and 3 not documented. In 3/35 episodes (9%) patients on treatment died during admission - none were related to IFI (2 died of AML; 1 of non-fungal pneumonia); all had possible IFI. Time to neutrophil recovery (> 0.5 x 10^3/L) in 20/35 episodes on treatment (57%) was ≥ 22 days (range 22-41, median 28): 10 had possible IFI, 2 probable, 6 unlikely and 2 were not documented. Summary/Conclusions. The EORTC/MSG IFI rate - possible/probable/proven - was only 22/137 (16%) episodes. This may be due to effective prophylaxis and/or early initiation of empirical treatment, but it also reflects the fact that the EORTC/MSG criteria were not intended for routine clinical use. Our audit data aims to allow regular review of anti-fungal policies, but is limited by its retrospective nature. Consequently, we have introduced prospective, continuous audit and will present our preliminary findings of the introduction of voriconazole as primary prophylaxis. Furthermore, we will outline a ongoing study combining galactomannan, PCR and measurement of inflammatory markers in blood, broncho-alveolar lavage and exhaled breath condensate for the early diagnosis of invasive aspergillosis.

References

FUNGAL INFECTIONS DIAGNOSTIC IN A NECROPSY STUDY. COMPARISON WITH THEIR CLINICAL SUSPICION

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Backgrounds. Intensive therapies on haematology illnesses treatment could open an increase on fungal infection in patients, especially, an aggressive medical profile. Samples that allows to establish the microbiologic or/and anatomopathologic diagnosis are not always easy to collect, and frequently an empiric treatment has to be started, based only on a suspect diagnosis. Aims. To compare the correlation between the suspicion of invasive fungal infection (IFI) and its clinical manifestations with the findings of the autopsy, in patient with malignant haematology diseases. Patients and Methods. We study 34 demised patients, 24 diagnosed patients of Acute Leukemia and 10 with other hematologic neoplasias that had been submitted to an autologous progenitor cell
transplantation. In 16 of the 34 patients the fungal infection was suspected at the beginning. According to the EORTC diagnostic criteria for IFI, 12 patients (75%), had a possible IFI and 4 cases (25%) presented a probable IFI. There were no cases with a proven IFI before death. Results. The autopsy demonstrated the presence of fungal infection in 10 patients: in 7 cases there was a clinical suspicion of fungal infection while in three cases there was no clinical suspicion. The autopsy revealed fungal infection in the lungs of 8 healthy volunteers and 10 MM patients were used for repeated stimulation of T lymphocytes. Activated T cells producing interferon γ were isolated using immunomagnetic separation (MACS) (Miltenyi Biotech) and expanded in vitro by phytohemagglutinin and high concentrations of interleukin 2. A specific cytotoxicity against myeloma cells was tested after the expansion of T cells to autologous or allogeneic myeloma. Activated T cells were labeled with CFSE. Allogeneic T cells and interferon γ negative fraction of T cells served as controls. Results. In an allogeneic setting with ARH 77 cells the enrichment of interferon γ positive T cells by magnetic beads in healthy donors started from a median of 2.8% (1.97-4.5%) to 48.57% (15.14-82.9%) after MACS and from 1.91% (1.14-3.4%) to 73.14% (3.9-88.75%) after MACS in CD3+ CD4 and CD3+ CD8 T cells, respectively. Interferon γ positive T cells were further expanded in vitro from 0.5×10⁶ to a median of 160×10⁶ (150×10⁶- 420×10⁶) T cells within 4 weeks and the test of cytotoxicity has demonstrated a high degree of specific killing of ARH 77 myeloma cells 69.17% (51.14-73.85%). Cytotoxicity against allogeneic myeloma was negligible. In an autologous setting with autologous myeloma cells used as an antigen, the enrichment of interferon γ positive T cells from MM patients started from 1.12% (0.27-6.2%) to 7.85% (0.42-12.6%) after MACS and from 1.9% (0.37-14.4%) to 14.7% (1.28-71.4%) after MACS in CD3+ CD4 and CD3+ CD8 T cells respectively. Interferon γ positive T cells were expanded in vitro from 0.12×10⁶ (0.05×10⁶-0.4×10⁶) to 88.5×10⁶ (35×10⁶-226×10⁶) within 8-12 weeks and the test of cytotoxicity has demonstrated only a modest specific killing of autologous multiple myeloma cells (18.88%) and allogeneic ARH 77 cells (18.21%). Conclusions. These data demonstrate a promising tumor-specific effect of allogeneic myeloma-reactive T cells but only a modest effect in an autologous setting in patients with MM. Whether that is due to a low MACS enrichment or low immunogenicity of autologous myeloma cells need to be further clarified.

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1253
CD40 LIGAND AND CALCIUM IONOPHORE TREATMENT OF DENDRITIC CELLS FROM HEALTHY DONORS AND PATIENTS WITH MONOCLONAL GAMMOPATHY OF UNKNOWN SIGNIFICANCE AND MULTIPLE MYELOMA
L. Kovarova, J. Michalek, M. Penka, R. Hajek
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Backgrounds. Dendritic cells (DC) are the most potent antigen-presenting cells that can initiate adaptive immune response. They differentiate from peripheral blood precursors and as an immature dendritic cells react to wide range of stimuli. Upon the activation/maturaton process they change their phenotypic, morphologic and functional characteristics. The ability to acquire and activate blood DCs makes them a valuable source for future immunotherapy trials, but there are inconsistent reports about the functional state of dendritic cells from patients with multiple myeloma (MM). Aims. Comparison of 48h treatment of immature dendritic cells with different stimuli as CD40 ligand (CD40L) and calcium ionophore (CI). Searching for differences in phenotype of DCs from healthy donors and patients with MGUS and MM after stimulation.

Methods. Ficol-Hypaque-separated peripheral blood mononuclear cells (PBMC) from 10 healthy donors and 12 patients (7 MM and 5 MGUS) were used. Adherent precursors of DCs were cultured with GM-CSF and IL-4. CD40L and/or CI were added in day 1 or 4 to generate mature DCs. Multicolor flowcytometric analysis was done in day 0 and after harvest of DCs in day 3 or 6. Following mononuclear antibodies were used: CD11c, CD80, CD83, CD86, lineage mixture, CCR2, CCR5, CCR7, IL-12, MIP-1a, HLA-DR. Results. The highest percentage of CD83, characteristic marker of mature DCs, was found in 3rd day of culture after stimulation CD40L and also CI. In the 6th day was the average percentage of CD83 up to the half of 3rd day. There was found no differences between donors and patients. Expression of HLA-DR was relatively constant, independent of the harvest time or type of the stimulation and again without differences between groups of patients and donors. Expression of costimulation molecule CD40L was obviously of expanded interst in maturation, because there were found no strong expression of CCR7, IL-12 and MIP-1a. We didn’t find significant differences between DCs generated from healthy volunteers and patients.

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1254
THE PREPARATION OF MYELOMA-SPECIFIC CYTOTOXIC T CELLS BASED ON INTERFERON γ PRODUCTION
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Backgrounds. Autologous hematopoietic stem cell transplantation has been considered recently as part of a standard treatment strategy in patients with multiple myeloma (MM). Here we attempted to enhance the immunotherapeutic potential of autologous T cells based on selection of myeloma-reactive lymphocytes in vitro. Aims. The aim of this study was to identify and characterize autologous myeloma-reactive select T cells in vitro and to evaluate their cytotoxic effect. Methods. Irradiated myeloma cell line ARH 77 or patient’s myeloma cells were used as tumor antigen and autologous dendritic cells in the autologous condition. The cell populations were shown by the autopsy to be affected by the fungal infection were: lung (9 cases), digestive (6 cases), heart (2 cases), kidney (2 cases), CNS (2 cases) liver (2 cases) spleen (1 case), mediastinum mass (1 case), and pancreas (1 case). It is relevant that in most patients, the organic involvement other that lung was not suspected before their death, and it was responsible for the very outstanding clinical manifestations during the end stage of the illness: superior vena cava syndrome (1 case), serious heart arrhythmias (1 case), profuse diarrhea (1 case), renal failure (1 case), and hepatic failure (1 case). Conclusion: Our study shows high incidence of clinical suspected IFI at the end-stage disease not confirmed with the autopsy, and the complexity of the clinical manifestations associated to this type of infections.
sequence determination can be used as a marker for evaluation of the vaccine strategy.

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1256
CRITERIA FOR CORD BLOOD DONOR SELECTION ON THE BASIS OF ROC CURVE ANALYSIS
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1Transfusion Centre, VALENCIA, Spain; 2Hospital Universitario La Fe, VALENCIA, Spain

The main limitation factor for a wide use of umbilical cord blood (CB) for transplantation is the cell dose. In this sense, many cord blood banks have set a total nucleated cells (TNC) content ranging from 60 to 100 x 10^7 as minimum required values for storing the units. In order to optimise cord blood banking and reduce the number of UCB units deferred before processing, an effort in donor selection is mandatory. Many authors have showed that placental and neonatal weight influence hematopoietic content of cord blood units. To establish obstetric criteria for selection of cord blood units before cryopreservation, in order to determine the optimal placental and neonatal weight for selecting cord blood donors according to the number of TNC, we have performed Receiver Operating Characteristic (ROC) curve analysis. ROC curve is a graphical technique commonly used to find optimal cut off value of a test using sensitivity and specificity data. We thought it could be useful to determine cut off values of placental and neonatal weight for an optimal selection of UCB units.

<table>
<thead>
<tr>
<th>TNC x 10^7</th>
<th>Cut-off</th>
<th>Area under the curve</th>
<th>95% confidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 x 10^7</td>
<td>Neonatal weight ≥ 3190</td>
<td>0.635±0.013</td>
<td>0.616-0.653</td>
</tr>
<tr>
<td></td>
<td>Placental weight ≥ 646</td>
<td>0.685±0.013</td>
<td>0.666-0.704</td>
</tr>
<tr>
<td>70 x 10^7</td>
<td>Neonatal weight ≥ 3195</td>
<td>0.682±0.012</td>
<td>0.619-0.656</td>
</tr>
<tr>
<td></td>
<td>Placental weight ≥ 645</td>
<td>0.692±0.012</td>
<td>0.662-0.701</td>
</tr>
<tr>
<td>80 x 10^7</td>
<td>Neonatal weight ≥ 3195</td>
<td>0.632±0.011</td>
<td>0.614-0.651</td>
</tr>
<tr>
<td></td>
<td>Placental weight ≥ 635</td>
<td>0.676±0.011</td>
<td>0.656-0.695</td>
</tr>
<tr>
<td>90 x 10^7</td>
<td>Neonatal weight ≥ 3195</td>
<td>0.611±0.011</td>
<td>0.612-0.649</td>
</tr>
<tr>
<td></td>
<td>Placental weight ≥ 635</td>
<td>0.648±0.011</td>
<td>0.629-0.686</td>
</tr>
<tr>
<td>100 x 10^7</td>
<td>Neonatal weight ≥ 3195</td>
<td>0.624±0.011</td>
<td>0.605-0.642</td>
</tr>
<tr>
<td></td>
<td>Placental weight ≥ 635</td>
<td>0.637±0.011</td>
<td>0.617-0.657</td>
</tr>
</tbody>
</table>

Results. We revisited 2590 cord blood units collected at Valencia Cord Blood bank for a four-year period. Mean TNC content of UCB before processing was 107.65±54.74 x 10^7. Mean neonatal weight and placental weight were 3313.36±319.5 kg and 652.2±122.1 g. ROC curve analysis was performed with MedCalc software for windows v. 7.4.2.0. Variable was considered 0 or 1 if TNC was < or ≥ 60, 70, 80, 90, and 100 x 10^7, respectively and classification variables were considered placental weight and neonatal weight. Results are shown on the following Table. We conclude this statistical analysis can be helpful to determine cut off value of placental/neonatal weight according to the required limit of TNC for each bank. This approach would reduce the number of collected units that are refused before processing.

1257
ANALYSIS OF THE CD34+ CELLS CONTENT OF THE CORD BLOOD UNITS STORED IN A REGIONAL CORD BLOOD BANK
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1Transfusion Centre, VALENCIA, Spain; 2Hospital De La Ribera, ALZIRA, Spain

Some clinical studies have shown that graft selection should be based principally on CD34+ cell dose and grafts should contain at least 1.7 × 10^6 CD34+ cells per kilogram of recipient’s body weight. However, criteria for selecting collections suitable for freezing and storage are not standardized. Although most banks have set a total nucleated cells (TNC) content ranging from 60 to 100 x 10^7 as initial minimum required values for storing the units, only a few banks select cord blood units on the basis of their CD34+ cell content. Aims. To analyse the CD34+ cell content of the cord blood units stored at the Valencia cord blood bank and the characteristics of the units according to their CD34+ cell content. We revisited the data of 2149 cord blood units stored at Valencia cord blood bank and selected on the basis of their TNC content (more or equal than 100 x 10^7). CD34+ cells were quantified by flow cytometry. CB sample was taken directly from the bag and after volume reduction before cryopreservation and 5 x 10^6 cells were incubated using monoclonal antibodies conjugated CD45 fluorescein and CD34 phycoerythrin (Becton Dickinson) and 7 amino-acetomin D as marker of DNA staining. Flow cytometric analysis was performed using Cell Quest software. ProCount progenitor cell enumeration kit was used in comparison with our standard protocol, giving similar results. Total CD34+ cells content was calculated by multiplying the CD34 percentage per TNC. A total of 2149 cord blood units were stored for a 5-year period. Mean TNC, CD34+ cell percentages and total CD34+ cells were 112.37±7.17 x 10^7, 0.63 ± 0.25% and 41.79±34.74 x 10^7, respectively. From these units, 489 (22%) had a total CD34+ cell content less than 20 x 10^7. Characteristics of the units according to their CD34 cell content are shown in the table. Conclusions. In order to increase the quality of cord blood units stored, the CD34+ cell content should be considered as a selection criteria of cord blood units for cryopreservation and storing.

<table>
<thead>
<tr>
<th>CD34+ Content x 10^6</th>
<th>N (%) of stored units</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20</td>
<td>489 (22.5%)</td>
</tr>
<tr>
<td>≥ 20</td>
<td>1660 (77.7%)</td>
</tr>
<tr>
<td>≥ 30</td>
<td>1218 (56.6%)</td>
</tr>
<tr>
<td>≥ 40</td>
<td>865 (40.2%)</td>
</tr>
<tr>
<td>≥ 50</td>
<td>610 (28.3%)</td>
</tr>
<tr>
<td>≥ 60</td>
<td>434 (20.2%)</td>
</tr>
<tr>
<td>≥ 70</td>
<td>292 (13.6%)</td>
</tr>
<tr>
<td>≥ 80</td>
<td>223 (10.3%)</td>
</tr>
<tr>
<td>≥ 90</td>
<td>160 (7.4%)</td>
</tr>
<tr>
<td>≥ 100</td>
<td>106 (4.9%)</td>
</tr>
</tbody>
</table>

Table 1.

1158
MESENCHYMAL STEM CELLS CONTRIBUTE TO THE HEALING PROCESS AND FUNCTIONAL IMPROVEMENT OF ISCHEMIC INJURED KIDNEY IN RAT MODEL
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Objective. Renal failure is a common disease with high morbidity and mortality. Ischemic injury is one of the most common causes of renal failure. Recent studies have reported that adult bone marrow-derived cells can contribute to renal remodeling and a dramatic repopulation of the mesangium. Moreover, there was a report that the role of bone marrow-derived hematopoietic stem cells in the regeneration of the renal tubular epithelium after ischemic injury in mice. When ischemic injury is inflicted on targeted organ, MSCs may migrate to the site of damage, undergo differentiation, and promote structural and functional repair. We evaluated whether bone marrow-derived MSCs contribute to the healing process and improve renal function in injured kidney of rat by ischemia. Materials and Methods. Right nephrectomy was performed in six-week-old SD rat. And the left renal artery and vein were clamped for 45 min followed by 2/3 nephrectomy was done and then clamp release to allow perfusion. MSCs prelabeled with green fluorescent protein (GFP) injected via tail vein. Peripheral blood was collected serially for evaluation of blood urea nitrogen and creatinine and functional evaluation was done with radiolabeled renal scan. Histologic study and confocal microscopic evaluation were performed at 4 days, 1 week, and 4 weeks after MSCs injection. Results. We demonstrated that GFP positive cells were detected in damaged kidney by confocal microscopy and engulfed MSCs promoted healing process by ischemic injury. Also engulfed MSCs differentiated into tubular epithelial cells, thereby restoring renal structure. In the group with MSCs injection, the levels of blood urea nitrogen and creatinine were lower than control group without MSCs injection (BUN Day 4, control group; 65.0±6.1, MSC infusion group; 51.1±5.1). And MSCs injected rats demonstrated that renal func-
tion recovered more rapid and more close to the normal value in radiosotope renal scan. Conclusions: The results presented here suggest that MSCs are capable of healing and functional restoring of damaged kidney by ischemic injury. So MSCs may be useful for cell therapy of renal failure.

1259
IMMUNOREACTIVITY TO ANTI-FIBRONECTIN AND ANTI-LAMININ POLYCLONAL ANTIBODIES IN PARAFFIN-EMBEDDED MICE BONE MARROW ARE DEPENDENT ON HISTOPATHOLOGICAL
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1Universidade Estadual de Londrina, LONDRA, Brazil; 2Universidade de So Paulo, SO PAULO, Brazil

Immunohistochemistry (IH) is an useful tool to study tissues and organs and it has been widely used in researchs or to supplement classical morphologic diagnosis including pathological conditions of the hematopoietic system. However, applicability of IH in bone marrow analyses presents some technical limitations, because some antigens are rendered during tissue processing (fixation, decalcification and paraffin embedding) making the applicability of this methodology unreliable. Bone marrow microenvironment contains cells of different tissues (bone, hematopoietic and stromal elements) and extracellular matrix (ECM) substances, mainly glycoproteins, proteoglycans and cytokines. The composition of bone marrow ECM is topographically variable and is associated to the development of different lineages of blood cells, suggesting that the existence of specific interactions between ECM, stem cells and stromal elements. In previous studies, we have showed that bone marrow from mice submitted to protein malnutrition underwent structural changes with decrease in cellularity and increase of glycoproteins extracted from ECM. The aim of this work was to evaluate the influence of fixative and time of fixation in the antigenic preservation of extracellular matrix glycoproteins fibronectin and laminin, and their distribution in bone marrow of mice. Estemum of well nourished Swiss mice, 2 to 3 months old, were fixed with 3 different fixatives: Methacarn (1 hour), 10% neutral buffered formalin pH 7.2 (1 hour, 6 hour or 24 hours) and 4% buffered paraformaldehyde pH 7.2 (24 hours). Decalcified using 5% nitric acid (3 hours) or 10% buffered EDTA, pH 7.2 (7 days) and then processed routinely with standard dehydration and embedding in paraplast. Tissue sections (5 micron thick) mounted on silane coated slides were dewaxed, rehydrated, and brought to phosphate buffered saline. Endogenous peroxidase activity was blocked by incubation for 30 minutes in 3% hydrogen peroxide. Sections were incubated with primary antibodies against fibronectin (1:400) and laminin (1:25) overnight at 0-4 ºC. After washes in PBS, slides were incubated with biotinylated secondary antibody for 30 minutes, washed in PBS and incubated with a streptavadin-biotin complex coupled to peroxidase for 30 min. Peroxidase activity was revealed with diaminobenzidine. Slides were counterstained with Harris hematoxylin. No immunoreactivity, for both anti-laminin and anti-fibronectin antibodies, was detected in any specimen fixed in 4% paraformaldehyde (24 h) and decalcified with EDTA. Sections fixed in Methacarn showed strong background reaction for laminin immunostaining and a false nuclear pattern for fibronectin immunostaining. Among the used conditions, adequate morphological and antigenic preservation of fibronectin and laminin were achieved on sections fixed in 10% buffered formalin during one hour and decalcified in 5% nitric acid. Tissue processing stages can significantly influence on immunoreactivity of antibodies against fibronectin and laminin. This way, sections fixed in 10% buffered formalin during one hour and decalcified in 5% nitric acid were selected to compare bone marrow ECM glycoproteins distribution in situ of nourished and malnourished mice.

Funding: Financial support: FAPESP, CAPES.

1260
VORICONAZOLE (VCZ) PROBABLY DOES NOT AFFECT THE PHARMACOKINETICS OF METHOTREXATE (MTX)
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1Regional Hospital, CESKE BUDEJOVICE, Czech Republic; 2University Hospital Motol, PRAGUE, Czech Republic

Background and aim: Both MTX & VCZ have many drug interactions. Whether an interaction exists between them is not known. The aim of the study was to explore whether oral VCZ affects the pharmacokinetics of MTX. Patients and Methods. With informed parental consent, a prospective study with standard clinical & drug monitoring was performed on 2 children with intermediate-risk, B-cell-precursor acute lymphoblastic leukemia during consolidation chemotherapy. This consisted of 6-mercaptopurine 25 mg/m2/d PO 56 d, MTX 2 g/m2/24h IV q 2 wk x4, leucovorin 15 mg/m2 x3 IV per each MTX & MTX 12 mg IT q 2 wk x4. The first child (case) has been on oral VCZ because of proven invasive pulmonary aspergillosis (IPA). The other (control) was infected free. MTX serum levels (µmol/L) were measured by fluorescence polarization immunoassay at 0, 18, 24, 36 & 42h of starting the infusion until achieving a concentration of ≤0.25. Inter- & intra-patient MTX levels were compared by Wilcoxon signed ranks test & Friedman test, respectively. Results. A 10.5-yr-old boy developed IPA, controlled by ABLC (29 d). Oral VCZ for 61 d was given thereafter (150 mg bid first d, then 100 mg bid 60 d). Consolidation started while being 17 d into VCZ treatment. Another 8-yr-old boy w/o IPA was undergoing identical consolidation under the same conditions. 3 pairs of MTX infusion running in parallel were evaluated. Baseline MTX levels were always below the detection limit (<0.05) in both. In the VCZ/MTX arm, MTX levels at 18, 24, 36 & 42h were, respectively: 25.62, 19.56, 0.52, 0.18 (1. MTX); 27.62, 24.17, 0.79, 0.25 (2. MTX); 23.13, 21.38, 0.46, 0.16 (3. MTX). The 4. MTX was delivered 5 d off VCZ, yielding a concentration of 21.53, 11.39, 0.32 & 0.11 at those time points, respectively. In the MTX-only arm, the corresponding figures were 24.14, 19.50, 0.62, 0.22 (2. MTX); 27.09, 18.63, 0.50, 0.23 (3. MTX); 26.60, 18.77, 0.50, 0.19 (4. MTX). All MTX levels were in the expected range, with non-significant differences (MTX PK changes are shown in table). However, while the intra-patient difference was not significant (p=0.1) in the control, it was so (p=0.02) in the case because of significant (p=0.02-0.04) depression in levels of the last MTX (off VCZ) vs the first 5 ones (on VCZ), within which the intra-patient difference was NS either (p=0.1). Within 1-3 d of every MTX infusion, the first pt developed cheilitis and photosensitivity over exposed body parts, the severity of which was related to intensity of sunshine. This reaction always resolved towards the next MTX. No other side effects were observed, nor IPA did exacerbate under VCZ/MTX. Assessment of VCZ levels is planned. Conclusions: Although the off-VCZ levels of MTX differed significantly from those on-VCZ in the case, this could be attributed to the well known intra-patient inter-dose variabilities in MTX disposition. The bulk of data suggests that oral VCZ seems not to affect MTX pharmacokinetics significantly. However, this should be confirmed on a larger number of pts and/or doses of MTX given during VCZ therapy.

1261
CELL DIFFERENTIATION AND APOPTOSIS OF U-937 LEUKEMIA CELL LINES BY A NEW COMPOUND FROM DENDROSTELLERA LESsertII
M. Mahdavi, R. Yazdaniparast
Institute of Biochemistry and Biophysics, TEHRAN, Iran

Recently, we have reported on the activity of 3-hydroxynedaphane- nine-3 (HK), purified from Dendrostellalesserti, to induce differentiation and apoptosis in HL-60 cells upon a single dose treatment at a drug concentration of 2.5-5.0 ng/ml during the relative potency of 3-HK compared to that of the crude extract, we looked for additional compound(s) with similar properties in the crude extract. Herein, we report on the isolation of a second and a more potent compound, with differentiation capability and apoptotic effects. The new compound inhibited growth and proliferation of U937 cells, with an IC50 of 1.75 µg/ml. The new compound could be confirmed on a larger number of pts and/or doses of MTX given during VCZ therapy.

1262
A TRIAL TO IDENTIFY SIDE EFFECTS OF DRUGS AT ULTRA-EARLY STAGE
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Matsumoto Living Cell Research Laborator, TOKYO, Japan

Method. Using the M-H Method, living blood cells are taken from a patient, and divided into two layers, upper and lower (i.e., ULRBC/U

haematologica/the hematology journal | 2006; 91(s1) | 453
and LLRBC(L) hereinafter referred to as U and I). Then, each layer of blood cells is put into 3 ml of RPMI-1640 solution and cultured in a 5% CO2 5%–CO2 incubator. Assuming that the entire amount of daily dosage of drug administered to the patient (g) is absorbed in 5 L of blood, the absorbed amount of drug in 3 ml of blood (X) is calculated as follows:

\[ X = \frac{mg \times \text{dose}}{\text{body weight}} \times \frac{\text{dose}}{\text{blood volume}} \times \frac{\text{blood volume}}{L} \times \frac{1}{5 L} \]

The calculated amount of drug (X) is added to 3 ml of physiological saline, and fully mixed together. The solution is then cleared of any undissolved drug by centrifugation. To the sterilized pipette, the solution is put into U and L, and cultured in a 5% CO2 5%–CO2 incubator. They are diachronically monitored with an inverted phase-contrast microscope, and recorded in photos and VCRs. U and L which the drug-dissolved solution is not added to are used as the control groups. Results. Compared with the control groups, if the drug is harmful for the red blood cells, RBC shows deformation and degeneration earlier, and dies after getting into the cells like firefly, ghost and black shell; i.e., the life of RBC is shortened. The WBCs (white blood cells) grow to enormous size in various complicated shapes, staying alive for three to five weeks. Moving Micro Livings and Mysterious chaos emerge in some cases. Compared with the control groups, Photo-Cytosis Phenomenon (named by Matsumoto) occurs sooner in some cases and later in other cases. Conclusions. If the proposed method is clinically adopted, individual variation against side effect of drugs can be determined easily. It would be secure and risk-free to check all of the drugs administered for possible side effects using the proposed method before administration. I believe medical science in the 21st century will go on in this direction. If the proposed method is started to use at the point of drug development, it can cut down on waste and expenses drastically.

1264
FIP1L1-PDGFRA POSITIVE CHRONIC EOSINOPHILIC LEUKEMIA IN TUNISIAN PATIENTS
S. Menif
Institut Pasteur, TUNIS, Tunisia

Hypereosinophilic syndromes (HES) are a heterogeneous group of rare disorders characterized by sustained and otherwise unexplained over-production of eosinophils with organ involvement and consecutive dysfunction. Detection of the FIP1L1-PDGFRα fusion gene or the corresponding cryptic 4q12 deletion in HES supports the diagnosis of chronic eosinophilic leukemia (CEL) and provides a molecular explanation for the pathogenesis of this disorder. We screened 11 Tunisian patients fulfilling the WHO criteria for HES for the presence of the FIP1L1-PDGFRα fusion gene using nested reverse transcription polymerase chain reaction on peripheral blood samples. 4 of the 11 patients (36%) were positive for this fusion gene. Sequence analysis revealed a substantial heterogeneity in the fusion transcripts due to the involvement of several FIP1L1 exons. All patients were male, the median age at diagnosis was 34 years (range, 18–50); one patient had a history of hypereosinophilia of more than 11 years. 2 patients had clinically important and symptomatic hypereosinophilic endomyocardial disease with thrombotic events. Splenomegaly was constant in FIP1L1-PDGFRα positive CEL but not in the other HES patients (only 1/7). Recent reports document the inefficacy of low doses of imatinib mesylate (100mg/day) in FIP1L1-PDGFRα+ CEL patients. All patients who present with sustained non reactive hypereosinophilia should be screened for the FIP1L1-PDGFRα fusion gene.

1265
TWO NEW MUTATIONS IN THE D1A1 GENE IN PATIENTS WITH RECESSIVE CONGENITAL METHEMOGLOBINEMIA TYPE I AND II
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Recessive congenital methemoglobinemia (RCM) is caused by deficiency of reduced nicotinamide adenine dinucleotide (NADH)-cytochrome b5 reductase (cyt b5r, EC 1.6.2.2). The cyt b5r gene (D1A1) is localised on chromosome 22q13-qter and contains nine exons; tissue-specific alternative transcripts originate 275-amino acid soluble and 800-amino acid membrane-bound isoforms. Cytb5r deficiency exists in two distinct clinical forms: in Type-I the enzyme deficiency is restricted to the red cell-soluble cyt b5r isoform and results in cyanosis; in Type-II the enzyme defect involves both the soluble and membrane-bound cyt b5r isoforms, and severe mental retardation and neurological impairment are also present. More than 35 different mutations of D1A1 gene have been reported in RCM: disruptive mutations (stop, frameshift, splicing) are associated with the severe Type-II disease. The aim of this study is to describe two cases of methemoglobinemia type I and type II respectively, associated with two new mutations of the D1A1 gene. The cyt b5r activity in the red cells was measured using the NADH-ferricyanide method. Molecular analysis of D1A1 gene was performed on blood DNA by PCR and direct sequencing. Case 1: the patient was a male infant of Northern Italian origin born at 38 weeks from spontaneous delivery. Cyanosis was noted at birth accompanied by reduced oxygen saturation (stable on 90-95%), but normal pO2 on arterial blood gas analysis and no evidence of cardiopulmonary abnormality. Weight at birth was 2710g and physical examination revealed neither developmental delay nor microcytic hypochromic anemia was detected. 26% of haemoglobin electrophoresis failed to demonstrate an M band. Studies on red blood cells revealed marked NADH-cyt b5r deficiency (0.9 IU/gHb; normal range 15.36-23.23.06 IU/gHb) while the parents exhibited levels of...
Acquired aplastic anemia (AA) is associated with poor response to IST resulted in a marked lymphohemopoietic recovery. Never-CD34-positive and HAE-9-positive erythroid cells were determined by flow-cytometry and DNA content analysis. Cell cycle analysis of CD34+ and HAE-9+ cell populations were studied by using the 7-amino actinomycin D (7AAD). As controls 14 healthy age-matched volunteers were studied. Results. The overall survival is 94% at the median follow-up of 30 months (range, 6-96 month). Complete or partial remission was achieved in 78% of AA patients in IST group. All transplanted patients are in complete remission. The numbers of CD34+ and HAE-9+ BM cells were decreased in most of untreated AA patients (3-fold below normal). In control group the mean number apoptotic CD34+ cells was 1% and 81% cells were in G0/G1 phase of cell cycle. In more than 50% of AA patients before treatment CD34+ progenitor cells were 2-3 fold more apoptotic. Also we revealed significantly greater proportion of CD34+ cells in S/G2/M phases of cell cycle. No significant difference was found in cell cycle state of committed HAE-9+ erythroid cells. The numbers of CD34+ and HAE-9+ cells rapidly increased after IST. Nevertheless increased apoptosis and cycling in CD34+ cell population persisted even in patients with complete remission. CD34+ cells compartment recovery was more complete in patients after alloBMT than after IST. The number of CD38+ CD8+ and CD8-DR+ lymphocytes significantly increased in BM during active phase of AA and returned to normal level at 6 month after IST treatment. However the number of CD8-DR+ lymphocytes increased again in most of patients after 12 month follow-up. Conclusion. Modern treatment modalities provide hematopoietic response and long-term survival in more than 80% of AA patients. Our data confirm that increased apoptosis and replicate stress in CD34+ cell compartment with profound stem cells deficiency correlate with signs of T-cell activation process in AA. Assessment of hematopoietic reservoir and immune-mediated pathology may provide additional information about remission status in AA patients.

**1267**

**IL-12 AND IL-10 PRODUCTION BY DENDRITIC CELLS (DC) FROM PATIENTS WITH APLASTIC ANEMIA (AA)**

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DCs accomplish determining function in antigen-specific T-cell immune response and antigen-specific self-tolerance development. Moreover, they yield either Th1 or Th2 commitment of naive lymphocytes due to immunoregulatory cytokines (IL-10 and IL-12) production. AA is characterized with increased Th1/Th2 ratio and altered immunoregulatory cytokine levels in BM. Therefore, the role of DC in the regulation of BM recovery is still under investigation. IL-12 and IL-10 secretion by DCs from patients with AA may provide additional information about cytokine production by BM DCs in patients with AA. Moreover, the cytokine production by BM DCs is supposed to be regulated by the cytokine production by BM DCs in patients with AA. Moreover, the cytokine production by BM DCs of AA patients in remission exceeded that of BM of healthy donors (184 vs 82 pg/106 cells, respectively). 36.4% (4/11) of AA patients exhibited increased baseline levels of IL-12 production by BM DCs compared to those of BM of healthy donors. These patients appeared to be resistant to standard scheme of IST and required continuous IST. Therefore, IL-12 and IL-10 production by BM DCs of AA patients is associated with resistance to standard scheme of IST.

**1268**

**INHERITED BONE MARROW FAILURE SYNDROMES IN LEBANON. PILOT DATA FROM THE LEBANESE PEDIATRIC HEMATOLOGY/ONCOLOGY GROUP**


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Backgrounds. Inherited bone marrow failure syndromes (BMFS) are rare disorders of the bone marrow with an increased risk of malignancy. Genetic features of these disorders are still being studied and could vary among different ethnic groups. Although some data is available from registries in the US and Europe, there is no such data from Lebanon. Aims. We aim to study the incidence, outcome and overall condition of patients affected with these disorders in Lebanon, as well as the genetic features of their families in order to establish a National BMFS registry. Methods. Patients with the following diagnosis were included: Amegakaryocytic Thrombocytopenia, Diamond-Blackfan Anemia (DBA), Dyskeratosis Congenita, Fanconi’s Anemia, Pearson’s Syndrome, Severe Congenital Neutropenia, Shwachman-Diamond Syndrome, Thrombocytopenia Absent Radii (TAR). Data collection sheets were filled by the pediatric hematologist for each patient diagnosed with any of these disorders between 1970 and 2006. Data was later entered into an Excel workbook and statistical analysis was performed. Results. Forty two (42) patients were identified so far. Fourteen had Fanconi anemia, nineteen had DBA, and six had severe congenital neutropenia, one had TAR syndrome, one had amegakaryocytic thrombocytopenia and one had Shwachman-Diamond Syndrome. Mean age was 10.2 years. At the time of data collection, 67% were alive and 33% were dead. Death was due to malignancy in 6 out
of 14 cases. DNA was available in 11 patients and was studied for possible mutations in the disease specific genes. Further results will be presented at the meeting. Summary/Conclusions. This is the first study looking at Inherited Bone Marrow Failure Syndromes in Lebanon. A registry has been created and is being updated constantly with new cases. A larger regional registry should be created with collaborative efforts and data should be compared among countries and then to registries in Europe and the USA in order to improve diagnosis and outcome of these patients and compare genetic determinants of these complex disorders.

1269 ALLOGENIC TRANSPLANTATION IN ADVANCED STAGES OF CML IN THE THIRD MILLENNIUM. IS THERE A DIFFERENCE ?
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Although since 2001 thyrosine kinase inhibitors have substantially changed the therapeutic approach in CML (chronic myelogenous leukaemia), its advanced stages still remain an important problem, in which transplantation is considered. Methods. We evaluated retrospectively 36 patients transplanted between the years 1992-2005 in the advanced stages of CML (i.e. further than 1st chronic phase). 20 of them proceeded the treatment in the nineties (non-imatinib era)(group 1), while 16 patients were transplanted in the last 5 years (group 2). In these patients imatinib was used either before transplantation (12 pts, 4 of them progressed despite this treatment), or after the transplantation (6 pts). Differences in disease stage when entering the transplantation, transplanted low or high mortality, overall survival, relaps rate and its response to further treatment were evaluated. Results. While in the group 1 there were only 25% of patients entering the transplantation in the reached chronic phase, the number increased to 44% in the group 2. The median of overall survival was 1.5 months in the group 1 compared to 16 months in the group 2. There are 3 surviving patients (15%) in the group 1 and 11 (66%) in the group 2. There was an enormous 100 days mortality in the group 1 (3 pts, i.e. 65%) compared to absent 100 days mortality in the group 2. Remission was achieved in 6 patients in the group 1, (3 of them relapsed later) and in 14 patients in the group 2, (6 of them relapsed later). DLI (donor lymphocyte infusion) or next transplant was used as a relaps treatment in 2 patients in the group 1 and in 5 patients in the group 2. In 6 patients of the group 2 imatinib was used after transplant. Conclusions. More feasible and probably less toxic achievement of further chronic phase by chemotherapeutic combinations with imatinib, better supportive care, earlier detection of minimal residual disease or relaps by molecular techniques, the use of imatinib for possible retransplantation in the non-imatinib era, are the main contributors for better survival in patients transplanted in the advanced stages of CML.

1270 INFECTION TRANSMISSION DURING GRAFT IN STEM CELL TRANSPLANTATION SYSTEM OF PREVENTION
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Backgrounds. The possibility of infection transmission by transplantation of cryopreserved blood stem cell concentrates is well known. For this reason EBMT (European Blood and Marrow Transplantation Group) and ISHAGE (International Society for Haemotherapy and Graft Engineering) standards include a panel of serological tests to be performed in donors and the patients with the aim to lower the likelihood of infection transmission. Aim. In the submitted paper attention is focused on different infection transmission by infusion of cryopreserved peripheral blood progenitor cells or bone marrow to the patient and/or cross contamination of stored grafts. Methods. After our preliminary investigations published 3 years ago the study was performed on a group of 35 related donors for allogeneic transplantation and 152 pts (mal. lymphoplastic, multiple myel., leukaemias, solid tumors, amyloidosis). They were tested before the peripheral blood stem cell or bone marrow harvest according to the standards of EBMT and ISHAGE-Europe: retroviruses (HIV,HTLV), hepatitis (A,B,C), herpes viruses (CMV, EBV, VZV, HSV), and ISHAGE standard. Results. Laboratory signs of active infection were found in 22 donors (62.85%) and in 91 patients (59.9%). The active infection from herpes viruses was the most common - in patients 50, in donors 21. Hepatitis B was found in only two cases. Conclusion: We can conclude that the rate of clinically unsuspected (but dangerous) infections in donors and patients remains relatively high in spite of the fact that the system of donor search and the whole transplantation procedure have improved in the last years. We confirmed that the developed system of safety assurance is extremely important and that the whole palette of preventive tests according to EBMT and ISHAGE remains fully justified.

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1271 EVOLUTION OF PATIENTS WITH ESSENTIAL THROMBOCYTOSIS (ET) TREATED WITH HYDROXUREA (HU) AND α-INTERFERON (IFN)
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Backgrounds. Essential thrombocytosis (ET) is a clonal myeloproliferative disease characterized by increased number of platelets, megakaryocytic hyperplasia and tendency to thrombosis and/or hemorrhage. The major aim of therapy is preventing thrombotic and hemorrhagic complications using cyto reduces agents that do not increase the risk of progression to acute leukemia or myelofibrosis. Aim of the study. To compare the results of the therapy with HU versus IFN in ET patients. Patients and Methods. 72 patients with ET followed between June 1999 - July 2005; median age 59,5 years (range 33 - 87 years); M/F: 32/40. Diagnostic criteria were those of the PVSG. 44 patients (median age 62 years) received HU, and 28 patients (median age 51 years) received IFN. HU dose varied according to platelet counts between 500 and 1500 mg/d. IFN dose was 9 MU/week. The purpose of therapy was to maintain platelet counts below 600.000/cmm and to prevent thrombotic or hemorrhagic complications. Patient with high risk for thrombosis received aspirine (75 mg/zi). Results. Patients treated with IFN presented a 90% response rate and those receiving HU had a 75% response rate. The reduction of platelet counts below 600.000/cmm was faster in the IFN group versus HU group (average 4 weeks vs 10 weeks respectively). The level of platelets during the treatment was maintained constantly around 400.000/cmm with IFN whereas in the HU group it was around 600.000/cmm. Thrombotic complications occurred more often in the IFN group - 8 cases (28,5%) with predominance of arterial thrombosis - 6 cases. In the HU group the incidence of thrombosis was 18,18% (5 cases - arterial thrombosis). The treatment with HU was better tolerated 6 cases with reversible leucopenia. The therapy with IFN was worse tolerated - 4 patients abandoned the treatment because of general symptoms. Conclusions. IFN was more effective in assuring a rapid and constant decrease of platelet number, but in the HU-treated group there was a lower incidence of thrombotic complications.

1272 CHRONIC MYELOPROLIFERATIVE DISORDERS: USE OF WHOLE BLOOD PLATELET LUMI-AGGREGOMETRY (WBPA) TO OPTIMISE ANTI-PLATELET THERAPY IN PATIENTS WITH PLATELET HYPERACTIVITY
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Twenty seven patients with chronic myeloproliferative disorders and in vitro evidence of platelet hyperactivity on WBPA studies (St Hema tol 199:105:618) were commenced on anti-platelet therapy comprising aspirin, clopidogrel and/or odourless garlic and the studies were repeated to assess the efficacy of the therapeutic agents. Only eight patients showed clear evidence of anti-platelet effect (inhibition of aggregation with arachidonic acid; aggregation and disaggregation with ristocetin), with the remainder showing the standard low dose (100 mg/d) use of DLI and an additional use of DLI (donor lymphocyte infusion). Our experience suggests that WBPA studies will not only enable selection of patients who will benefit from anti-platelet therapy but also assess the efficacy of such therapy.
PREVALENCE OF THE ACQUIRED MUTATION V617F ON THE JAK2 GENE ON THE DIFFERENT MYELOPROLIFERATIVE DISORDERS, AND ITS CONTRIBUTION TO A CORRECT DIAGNOSIS


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Backgrounds. The molecular basis for Ph-Chronic Myeloid Disorders (CMPD) remain widely unknown, although nowadays, mutations on the JAK2 gene are thought to play a role in its pathogenesis. The Janus Kinase 2 gene (JAK2) is a tyrosin kinase involved in the transduction of cell proliferation stimuli. Unlike healthy patients, the JAK2 point mutation, 1849G→T which produces the substitution of Phe for Val (V617F) on the resulting protein, has been identified on many CMPD cases. 

Aims. To study the prevalence of the point mutation V617F on JAK2 on patients suffering from CMPD, and to determine its potential contribution for the classification and final diagnosis in these cases. Methods. A total of 99 patients were studied, which distributed as follows: 16 cases of reactive myeloid cytoses, 14 suspected CMPD, and 69 confirmed CMPD. Within the 14 suspected there were: 5 chronic idiopathic myelofibrosis (CIM), 14 Polycitemia Vera (PV), 28 essential thrombocythemia (ET), and 22 mixed CMPD. The screening for the JAK2 mutation was performed on samples obtained from bone marrow aspirates, or peripheral blood, using PCR according to E. Joanna Baxter technique (Lancet 2005). Results. From the total of patients studied (99), 53 were JAK2+ (53.5%), and distributed as follows: 3 on the group diagnosed as reactive myeloid cytoses (18.7%), 4 on the non confirmed CMPD (26.6%), and 46 on the CMPD (66.7%). The JAK2 mutation’s prevalence on the different subgroups of CMPD was: 15 (53.6%) of 28 for ET, 13 (92.8%) of 14 cases of PV, 14 (60.0%) of 5 cases of CIM, and 15 (62.2%) of 22 patients with mixed CMPD. Conclusions. JAK2 mutation is frequently detected in the Taiwanese patients with myeloproliferative disorders as in the western patients, which should be used as a diagnostic tool in the future routine hematological practice.

EPOR MUTATIONS IN FAMILIAL CONGENITAL POLYCYTHEMIA VERA

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Haematology Clinic, IOANNINA, Greece; Laboratory of Genetics Unit, IOANNINA, Greece; Laboratory of General Biology, IOANNINA, Greece

Backgrounds. Primary familial congenital polycythemia (PFCP) is a rare myeloproliferative congenital and dominant disorder. It is caused by inherited defects in hypoxia sensing or by inherited vital defects in red blood cell precursors that cause augmented responsiveness to Epo. These facts result in isolated proliferation of the erythroid progenitor cells and in erythrocytosis. The Epo-receptors (Epo-R) is located on the surface of erythroid cells. The Epo-R gene is situated on chromosome 19. Genetic changes of the Epo-R gene have been related to the pathogenesis of PFCP. Specifically, twelve mutations in patients with PFCP have been recognized. Aim of this study was to investigate the presence of Epo-R mutations in patients with PFCP. Methods. We studied eight families (20 individuals) with PFCP of Greek origin. All individuals had Ht>53% and their age range was between 16 and 58 years. Genomic DNA was extracted from peripheral blood lymphocytes, according to standard procedures. SSCP and sequencing analysis was performed to detect mutations in exon VIII of the Epo-R gene that previously has been related to PFCP. Results. No mutation was identified in our patients which could underline the molecular defects of the PFCP. Conclusions. Our results can lead to the conclusion that the molecular cause of familiar polycythemias in Greek patients cannot be attributed to sequence alterations in exon VIII of the Epo-R gene.

HLA ASSOCIATIONS WITH CHILD’S AND ADULT’S ACUTE LYMPHOCYTIC LEUKEMIA

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Background. Acute lymphoblastic leukemia (ALL) has two rising of morbidity: in children of 2-4 years and in adults after 50 years. The aim of our study was to identify the associations between HLA and ALL in children and in adults in our population.

Methods. HLA-A, -B, -Cw -DRB1 typing was done in 26 children with ALL (the median patient age was years 5.6; range, 1-13 years) and in 42 adult patients with ALL (the median patient age was 52.6 years; range, 16-52 years). 328 healthy donors of blood components (the median age was 29.9 years; range 15-59 years) were control group. The HLA frequencies were counted and compared by exact Fisher’s test. The strength of association between HLA and disease was determined by the evaluation of relative risk (RR). Results. Children with ALL had significantly increased frequency of DRB1*07 (46.2% vs. 26.8% in control group, see the table below), RR of child’s ALL for DRB1*07 carriers was 2.3. Adults with ALL had significantly increased frequency of DRB1*11 (58.1% vs. 22.6% in control group). RR of adult’s ALL for DRB1*11 carriers was 2.1. The frequency of DRB1*01 was significantly decreased in both groups: in children with ALL (7.7%) and in adults with ALL (11.9%) in comparison with controls (26.2%). RR of child’s and adult’s ALL for DRB1*01 carriers in our population was 0.23 and 0.38 respectively. In conclusion, it seems that DRB1 gene may be involved in predisposition and resistance to ALL development both in children and in adults.

Table 1. DRB1 frequencies in children and adults with ALL

<table>
<thead>
<tr>
<th>DRB1</th>
<th>Children</th>
<th>Adults</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=26</td>
<td>n=42</td>
<td>n=328</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td>DRB1*01</td>
<td>7.7</td>
<td>11.9</td>
<td>26.2</td>
</tr>
<tr>
<td>DRB1*07</td>
<td>46.2</td>
<td>21.4</td>
<td>26.8</td>
</tr>
<tr>
<td>DRB1*11</td>
<td>34.6</td>
<td>38.1</td>
<td>22.6</td>
</tr>
</tbody>
</table>

Methods. HLA-A,-B,-Cw,-DRB1 typing was done in 26 children with ALL (the median patient age was years 5.6; range, 1-13 years) and in 42 adult patients with ALL (the median patient age was 52.6 years; range, 16-52 years). 328 healthy donors of blood components (the median age was 29.9 years; range 15-59 years) were control group. The HLA frequencies were counted and compared by exact Fisher’s test. The strength of association between HLA and disease was determined by the evaluation of relative risk (RR). Results. Children with ALL had significantly increased frequency of DRB1*07 (46.2% vs. 26.8% in control group, see the table below), RR of child’s ALL for DRB1*07 carriers was 2.3. Adults with ALL had significantly increased frequency of DRB1*11 (58.1% vs. 22.6% in control group). RR of adult’s ALL for DRB1*11 carriers was 2.1. The frequency of DRB1*01 was significantly decreased in both groups: in children with ALL (7.7%) and in adults with ALL (11.9%) in comparison with controls (26.2%). RR of child’s and adult’s ALL for DRB1*01 carriers in our population was 0.23 and 0.38 respectively. In conclusion, it seems that DRB1 gene may be involved in predisposition and resistance to ALL development both in children and in adults.
INTERMEDIATE ANALYSIS OF THE SPANISH QUIT REGISTRY FOR PATIENTS WITH ACUTE LEUKEMIAS AND NON-HODGKINS LYMPHOMAS TREATED WITH INTRATHecal CHEMOTHERAPY

QUIT study, BADALONA, Spain

Background and Aim. CNS involvement in pts diagnosed with hematological malignancies is an unfrquent complication that carries poor prognosis. The indication and the schedules of prophylaxis and treatment of CNS involvement in AL and NHL are not homogenous within countries and within the same country. The aim of the QUIT study was to report the practices of CNS prophylaxis and treatment in patients with AL and NHL in Spain. Methods. Prospective study conducted from June to December 2005. Adult pts (≥18 yr.) diagnosed with hematological malignancies who received IT chemotherapy as CNS prophylaxis or treatment were consecutively included through online registration. Results: 242 pts from 27 hospitals were included. Mean (SD) age 48 (16) yr. and 133 (55%) males; 142 had AL and 100 NHL. 1. AL patients: 85 ALL and 57 MCL. CNS therapy: 17 cases, at diagnosis and 6 as relapse (5 isolated, 1 combined). IT therapy consisted of triple IT therapy (TTT, MTX+ARAC+Hydrocortisone) in 14 and liposomal depot cytarabine in 3 pts. CNS prophylaxis: 125 patients and consisted of TIT in 104 (83%), and MTX IT in 19 (15%), IT ARAC in 1 (1%) and 1 IT liposomal depot cytarbine (1%). No cranial irradiation either for prophylaxis or therapy was given in any case. 2. NHL patients: 56 diffuse large B-cell, 18 Burkitt’s, 5 follicular, 5 mantle cell, 10 T cell, and 6 other subtypes; stage IV 70%, B symptoms 52%, bulky disease 51%, extranodal involvement 79% (bone marrow 45%) and >1 extranodal site 44%, increased LDH levels 64%, IPI score 3 68%. CNS therapy: 24 pts, 16 at diagnosis and 8 as first (5) or subsequent relapses (3). CNS therapy consisted of TIT in 15/24 patients (62%), IT liposomal depot cytarabine in 8/24 (33%) and MTX IT in one (4%). CNS prophylaxis was given to 76 pts, the main reasons for administration were extranodal involvement (58%), aggressive histology (46%), high LDH levels (46%), IPI score ≥3 (31%), bulky disease (26%) and HIV infection (10%). IT prophylaxis consisted of TIT in 88% followed by MTX IT in 9% and IT liposomal depot cytarabine in 3%; Cranial irradiation was administered in 3 cases (2 as therapy and 1 as prophylaxis). Conclusions. In clinical practice in Spain the patterns of CNS prophylaxis and therapy for AL are homogenous. For NHL there is heterogeneity of indication of prophylaxis. TIT was the most frequent schedule for CNS prophylaxis and therapy in AL and NHL. It is of note the administration of new drugs i.e.: liposomal depot cytarbine for CNS therapy and prophylaxis in NHL and AL, and the lack of use of cranial irradiation.

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EXPRESSIoN OF ZAP-70, CD38 AND IgVH MUTATIONAL STATUS AS PREDICTORS OF TREATMENT IN BINET STAGE A CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: a combination of Zap-70/CD38 expression has been proposed as a predictor of treatment in CLL patients by several authors. Aim: to analyse the predictive value of this model in a series of 73 patients consecutively diagnosed of B-CLL at our institution from 1997 until 2005, whose all three variables were available. Methods. Zap-70 and CD38 expression was analysed by flow cytometry and IgVH mutational status by direct sequencing with 98% cut off. All 73 patients were in Binet stage A. Median age was 66 years (54 to 85), male sex 40 (50%). ZAP-70 and CD38 expression cut off were 20% for both antigens. Median follow-up was 56 months (6.7 to 134).

Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>N(%)</th>
<th>Time to treatment p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD38</td>
<td>&lt;20%</td>
<td>48 (66)</td>
</tr>
<tr>
<td></td>
<td>≥20%</td>
<td>25 (34)</td>
</tr>
<tr>
<td>ZAP-70</td>
<td>&lt;20%</td>
<td>44 (60)</td>
</tr>
<tr>
<td></td>
<td>≥20%</td>
<td>29 (40)</td>
</tr>
<tr>
<td>IgVH</td>
<td>mutated</td>
<td>40 (55)</td>
</tr>
<tr>
<td></td>
<td>unmutated</td>
<td>33 (45)</td>
</tr>
<tr>
<td>ZAP-70/IgVH</td>
<td>positive/unmutated</td>
<td>21 (29)</td>
</tr>
<tr>
<td></td>
<td>discordant</td>
<td>20 (27)</td>
</tr>
<tr>
<td></td>
<td>negative/mutated</td>
<td>32 (44)</td>
</tr>
<tr>
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<td>CD38/IgVH</td>
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<td>14 (19)</td>
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<tr>
<td></td>
<td>negative both</td>
<td>39 (53)</td>
</tr>
</tbody>
</table>

NR: not reached.

Results. All 3 variables provided significant prognostic information with respect to the need of treatment. An intermediate prognostic group was identified for discordant cases. The predictive value of the three dual combinations is detailed in the table above. Concordant positive expression of CD38/ZAP-70 identifies an aggressive group of patients with the shorter time to first treatment (39 months versus not reached) and the lower percentage of discordant cases (19%). CD38/ZAP-70 positive expression predicts an unmutated status of IgVH gene in 75% of the cases whereas negativity for both antigens predicted a mutated status in 85% of cases. In the IgVH mutated group (n=40), 3 out of 11 patients treated were discordant cases who co expressed both antigens. Conclusion: in Binet A stage CLL patients, CD38/ZAP-70 positive expression allows to identify a group of patients with a shorter time to first treatment (39 months) in discordant cases without the knowledge of the mutational IgVH gene status.

LONG-TERM RESULTS OF THALIDOMIDE IN REFRACTORY AND RELAPSED MULTIPLE MYELOMA WITH EMPHASIS ON RESPONSE DURATION

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Hospital Clinic, IDIBAPS, BARCELONA, Spain; Hospital Clinic, IDIBAPS, BARCELONA, Spain

Background. Thalidomide administered as a single agent produces a response rate of about 40% in patients with refractory or relapsed multiple myeloma (MM). However, the data on the duration of such responses is limited. Aim. The aim of our study was to determine the
quality and duration of responses to thalidomide in patients with refractory or relapsed MM. 

Patients and methods. Forty-two consecutive patients (22M/20F; median age: 61 years) with refractory (20) or relapsed (22) MM were given thalidomide as single agent at our institution from November 1999 to December 2003. Most of the patients (70%) had previously received 2 or more lines of therapy, and 36% had undergone autologous stem cell transplantation (ASCT). The drug was initially administered at a daily dose of 200 mg and later increased, depending on tolerance, by 100 to 200 mg every two weeks up to a daily dose of 800 mg. In responding patients, the dose of thalidomide was thereafter progressively tapered to a maintenance dose of 100 mg/day. No prophylactic anticoagulation was given. Results. Eighteen patients (43%) responded to thalidomide: 11 minimal responses (MR) and 7 partial responses (PR) according to the EBMT criteria. The median time to response was 3 months and the median duration of therapy in responding patients was 9 months. The reasons for discontinuing thalidomide in responding patients: toxicity in 10 cases, progression in 4, and death due to pneumonia with respiratory failure in 2. In 2 additional patients treatment was stopped at the time when they were intensified with ASCT. The toxicity mainly consisted of peripheral neuropathy and fatigue. At the time of this analysis, all responding patients had progressed except one who remains in continued stable PR for more than six years after starting thalidomide therapy and for 3.5 years after thalidomide discontinuation. The median time to progression was 15.6 months (range 1.3-70+), with a trend towards a longer duration for patients who achieved PR vs. MR (21.2 vs. 11.2 months, p=0.11). The median duration of response was 12.4 months (range 0.5-67+; 17.2 months for PR vs. 9.7 months for MR, p=0.11). Conclusion. These results show that the effect of thalidomide in refractory/relapsed MM can be sustained, particularly in patients who achieve a good response, and support the investigation of this drug as maintenance therapy.

Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Men 11.4±5.8μmol/L</th>
<th>Men 11.3±2.94μmol/L</th>
<th>Men 11.2±2.4μmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women 8.9±8±2.13</td>
<td>Women 8.8±1±2.52</td>
<td>Women 14±1±7.5</td>
</tr>
</tbody>
</table>

If we exclude those women with cardiovascular disease who had higher homocysteine’s plasma levels from the other groups, significant differences among the levels of the others were not noticed. Thus many questions remain to be answered before the effect of Thcy is finally considered as a risk factor of cardiovascular disease or stroke. Many more studies are needed before we are able to focus our attention in therapy and prevention.

1282

ANALYSIS OF THE EFFECTIVE AGENTS IN DEVELOPMENT OF FACTOR INHIBITOR IN HEMOPHILIC PATIENTS EVALUATION OF 445 PATIENTS IN CENTRAL PART OF IRAN

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Backgrounds. In hemophilia the development of inhibitors is a serious problem. Inhibitors reduce the efficacy of haemostatic treatment and clearly cause additional morbidity. Understanding the effective agents in arising factor inhibitors could be helpful in management of hemophilic patients. Aim: To evaluate the development of inhibitors and analyze the effective agents in it, in patients with hemophilia. Method: A comprehensive study underwent in Hematology-Oncology Department- Ishafan University of Medical Sciences. All hemophilic patients (445 patients) underwent frequent testing for inhibitors and the development of an inhibitor was defined by a titer > 0.5 Bethesda units (BU)/ml in any sample. Clinical History, Laboratory and treatment chart of patients were studied in January 2006. Results. From 401 men and 44 women with factor deficiency with Mean±SD age of 23.25±13.15, 27 patients (6.06%) showed factor inhibitor. The mean duration between diagnosis of disease and inhibitor arising was 15.26 years. From them 26 had Factor (F) VIII deficiency who were 7.6% of patients with FVIII deficiency, and one had FIX deficiency. According to forward stepwise logistic model (with percentage correct of 95%) treatment with factor concentrates with 2.9 times, and FVIII deficiency with 12.7 times chance correlate with factor inhibition. Other agents like, severity of disease, blood group and age of patients do not enter in the model. In this study 12.4% of patients who used factor concentrates, developed factor inhibitor (0.001). Conclusion: These data provide estimates of the rate of inhibitor in factor deficiencies. Beside several advantages of factor usage in treatments of hemophilic patients, the more chance of coloration between inhibitor development and it should be noticed.

1283

MANAGEMENT OF LIFE THREATENING SEVERE HEMORRHAGES AND UNSAFE INTERVENTIONS IN NON-HEMOPHILIC CHILDREN BY RECOMBINANT ACTIVATED FACTOR VIIa (RFVIIa)

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The literature on the use of recombinant factor VIIa (rFVIIa) that was initially used in hemophilic patients with inhibitors, for hemorrhages that cannot be managed with conventional methods or operations that cannot be performed safely is increasingly growing. Here we present a group of non-hemophilic patients with hemorrhagic problems or hemorrhage risk for some interventions that were successfully resolved with the use of rFVIIa. The patient group was composed of 20 patients with different disorders resulting in similar results as hemorrhage or hemorrhage risk. Most of the patients were diagnosed as primary or secondary liver disorders. The remaining cases were patients with leukemia, sepsis, intracranial hemorrhage, and burns. Some of the patients had multiple problems like a patient with liver disorder and intracranial hemorrhage or a leukemia patient with sepsis and DIC. rFVIIa had been administered to the patients at dosage between 70-150 microg/kg up to 6 doses with 2-3 hours intervals. All of the patients had benefited from the use of rFVIIa even though some of them ceased as a result of primary disease. As in our experiences, rFVIIa can be safely used in high-risk patients with a history of severe hemorrhage, for whom no improvement can be achieved in the hemostasis tests. We conclude that rFVIIa is effective in the control of life threatening hemorrhage in pediatric patients.

1284

HEMARTHROSIS AS COMPLICATION OF SUPERWARFARIN POISONING

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Poisoning with long-acting anticoagulants is known to lead to disturbance of hemostasis. Such haemorrhagic complications, like the ones after poisoning with rodenticide, are often difficult to identify. To present, for the first time in literature, a case of haemarthrosis as a haemor-
In contrast the disposition of platelets to get activated by collagen in autologous setting and the totality of aggregation was significantly lower in A-PC on d0 and d2 compared to T-PC (values as mean±SD; *p<0.05; **p<0.01). Conclusion: Standardised PC-collection in MCC is effective and well tolerated as expected and there are no differences in storage parameters in comparison to platelet collection only. Preparation of plt by the AMICUS cell separator leads to a higher activation level of plt and therefore to a reduced ability to be further activated to aggregate in vitro. This fact may be due to the different plt collection modality. In the AMICUS device plt are centrifuged highly concentrated until the end of apheresis and resuspended in plasma afterwards contrary to the TRIMA Access, where plt rich plasma is collected outside the cell separator. However, the in vivo relevance of our findings has to be evaluated in clinical observations.

1285

PLATELETS COLLECTED BY MULTICOMPONENT APERESIS WITH TWO DIFFERENT DEVICES: ACTIVATION MARKERS AND FUNCTION IN AGGREGOMETRY DURING SEVEN DAYS OF STORAGE

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Introduction. The collection of red blood cells (RBC) and platelets (plt) by multicomponent collection (MCC) is a mean to reduce donor exposure of polytransfused patients. We compared plt activation and plt function parameters in platelet concentrates (PC) collected by two different devices during 7 days storage to estimate a possible association between level of activation and collection modality as well as maintained platelet function. Materials and Methods. Fifteen donors, each with two donations, were included in our study. For each donor we used the TRIMA Access (Gambro BCT) (T-PC) and AMICUS (Baxter) (A-PC) with an interval of at least two months in between. PC were stored under agitation at 22±2°C and were tested on day 0, day 2 and 7 for platelet aggregation parameters and markers CD62P, CD63 and thrombospondin binding (TSP) by flow cytometry (FACSCalibur, Becton Dickinson). Additionally, aggregometry was performed using collagen as agonist (BCT, Dade Behring) and maximum aggregation (MA, %) and maximum velocity of aggregation (Vmax, mE/sec) was measured. Results. Per apheresis a double PC and one unit of packed RBC (250ml) were collected. Mean plt concentrations in T-PC (1.407±103×10^10/L) and A-PC (1.291±228×10^10) were comparable (p=0.09). As shown in the table we found statistically significant higher percentages of CD62P- (d0 and d2), CD63- and TSP- (d0, d2 and 7) expressing plt in A-PC compared to T-PC.

Table 1.

<table>
<thead>
<tr>
<th>CD62p</th>
<th>CD63p</th>
<th>TSP</th>
<th>MA</th>
<th>Vmax</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>mE/sec</td>
</tr>
<tr>
<td>d0 T-PC</td>
<td>17.3±6.1</td>
<td>19.2±9.4</td>
<td>11.5±5.1</td>
<td>86.7±5.3</td>
</tr>
<tr>
<td>A-PC</td>
<td>62.6±15.5**</td>
<td>36.2±16.5**</td>
<td>25.2±7.1**</td>
<td>88.9±5.6**</td>
</tr>
<tr>
<td>d2 T-PC</td>
<td>36.2±12.6</td>
<td>34.9±12.5</td>
<td>17.3±6.1</td>
<td>81.7±3.6</td>
</tr>
<tr>
<td>A-PC</td>
<td>60.7±20.9**</td>
<td>62.1±20.9**</td>
<td>26.0±9.2</td>
<td>56.7±20.5**</td>
</tr>
<tr>
<td>d7 T-PC</td>
<td>67.5±8.7</td>
<td>63.8±18.3</td>
<td>21.4±7.5</td>
<td>34.1±7.6</td>
</tr>
<tr>
<td>A-PC</td>
<td>75.2±12.1</td>
<td>69.2±19.2**</td>
<td>28.9±9.6**</td>
<td>16.3±24.4</td>
</tr>
</tbody>
</table>

1286

BACTERIAL CONTAMINATION OF APERESIS PRODUCT FOR AUTOLOGOUS PERIPHERAL BLOOD TRANSPLANTATION AND THEIR CORRELATION WITH POST-TRANSPLANT BEHAVIOUR

S. de Hematología, H. V. de la Artixaca, MURCIA, Spain

Peripheral blood progenitors cells (PBPCs) for autologous transplantation require extensive manipulation in several steps. Bacterial contamination is a well known risk in most of the transplant units, although their clinical significance remains controversial. We reviewed the clinical records of our transplanted patients. The aim was to know the incidence of bacterial contamination in PBPCs, and how this affects post-transplant infections, time to engraftment, transfusion requirements, days of fever and hospitalization of our patients. A total of 154 transplanted patients received 525 aliquots of PBPC products, a median of 3 (1-20) per patient. 127 patients (95%) had fever in the post-transplant period; 117 (92%) of them had positive blood cultures obtained from peripheral vein or central venous catheter. The most frequent bacteria were: coagulase-negative Staphylococcus in 53% patients, followed by coagulase-positive Staphylococcus in 3%, E.coli in 3%, Streptococci in 3%, and others in less than 1%. Bacterial contaminated PBPCs were infused to 11 patients. The bacteria isolated from these aliquots were: 2 Streptococcus viridans, 5 coagulase-negative Staphylococcus, 2 Corynebacterium and 2 no identified Gram-positive bacillus. These patients received no prophylactic antibiotic therapy, but at the moment of infusion peripheral blood granulocyte counts were normal. In three out of these eleven patients receiving contaminated PBPCs, the same bacteria was isolated in blood. No difference was found between patients receiving grafts with and without contaminated PBPCs in terms of days of fever (6±1-7 vs 4±1-25), transfusion requirements, days of hospitalization, days of engraftment of granulocytes (12±10-19 vs 11±9-25), and platelets (13±10-25 vs 12±8-35). In the group of patients receiving contaminated PBPCs, no difference was found between the three patients with the same bacteria and the eight with a different one in terms of: days of hospitalization, days of fever (4±1-6) vs 3±2-7 respectively, day of granulocyte 12±10-12 vs 12±10-18) and platelets engraftment 11(10-15) vs 13(12-21) respectively. From our experience, it seems that the infusion of contaminated hematopoietic cells has not clinical translation although there are few cases. The microorganism most frequently isolated in the contaminated PBPCs aliquots and in the blood cultures of patients with fever was S. epidermidis. As this bacterium is frequently associated with vascular catheter infections, we cannot know if the contamination is due to infused product or not.

1287

THERAPEUTIC CYTAPHERESIS: AN ADAPTED STRATEGY FOR LEUKODEPLETION

1IRCCS Policlinico San Matteo, PAVIA, Italy; 2IRCCS Policlinico San Matteo, PAVIA, Italy

Background and Aims. Hyperleukocytosis may represent a life-threatening condition for patients with ALL, AML, CLL or CML. Elevated white blood cells counts may cause acute respiratory distress syndrome, intracranial bleeding and the tumor lysis syndrome after chemotherapy. Therapeutic leukapheresis dramatically decreases the number of circulating leukocytes with beneficial effects on hyperviscosity and leukostasis.

rhagic complication of warfarin poisoning. A 67-year-old man was admitted to our clinic with melena, epistaxis and haemorrhaxis in his left knee. He received portion of rodenticide substance 15 days ago. 2 days after the reception he was hospitalized for 6 days with melena and epistaxis. Screening tests of haemostasis revealed undetermined PT (INR) and aPTT. Initially, he was treated with 4 units of fresh frozen plasma (FFP) and 5×10 mg of Vitamin K iv per day, for 6 days. For further investigation he was admitted in our unit, where, during the first 7 days of hospitalization, had also melena. We administrated supporting treatment (FFP and 6 units of red cells) and we successfully treated epistaxis with topical haemostasis. Haemorrhaxis was treated with FFP (4 unitsx2 per day, for two months). Initial screening: 1st day: INR=7.15, aPTT=79.9%, PT=107.8%, aPTT = 95%, Hct= 25.8%. After the third day, values of INR varied from 2.04 to 4.76. PT from 15.5’ to 44.7’ and aPTT from 35’ to 75.7’. After the 11th day the Hct remained stable at 34%. During the 23th day we administered 1X10 mg Vitamin K iv and three days later 5X10mg iv. Screening test at 26th day showed: INR=1.19, PT=15.2’, aPTT=37.5’, Hct=52%. Immunological and biochemical tests, tests of electrolytes, complete study of haemostasis, microscopic examination of excrements for fat and undigested fibers, tests for viruses and complete study of liver function (for latent hepatic insufficiency) were performed. We found very low levels of vitamin K dependant factors (II, VII, IX, X, protein C and S), which were normalized after the administration of FFP and Vitamin K. Levels of the rest of the factors were normal. Prothrombin times in aroo and bulk, and 2nd section of duodenum were revealed endoscopically, while the psychiatric estimation revealed a disturbed personality. Acquired disturbances of haemostasis after poisoning with warfarin (rodenticides) substances were described in several cases and have often led to death. This is why a long duration treatment and follow-up are required. Haemorrhaxis as a complication of warfarin poisoning is presented for the first time in literature.
sis. Moreover leukocytoreduction prior to chemotherapy can reduce metabolic and renal complications due to rapid cellular lysis. Despite benefits, leukocytoreduction may pose multiple concerns because of patient’s poor clinical conditions like concomitant anaemia, thrombocytopenia and hypertension. ASFA Committee classifies hyperleukocytosis as category I indication for therapeutic apheresis, nevertheless the effect is only temporary and the institution of appropriate chemotherapy is essential. We report the experience of performing leukocytoreduction at our Apheresis Service employing a third generation cell separator adapted on the basis of target cells separation characteristics. Methods. a summary of the patients characteristics is given in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Characteristics of the patients (n=16).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female*</td>
</tr>
<tr>
<td>Age (years)*</td>
</tr>
<tr>
<td>Diagnosis:</td>
</tr>
<tr>
<td>ALL (6%)</td>
</tr>
<tr>
<td>MDS (19%)</td>
</tr>
<tr>
<td>NHL with circulating blasts (1%)</td>
</tr>
<tr>
<td>AL in MDS 1 (6%)</td>
</tr>
<tr>
<td>Peripheral blood values:</td>
</tr>
<tr>
<td>WBC (×10⁹/microL)*</td>
</tr>
<tr>
<td>Hb (g/dL)*</td>
</tr>
<tr>
<td>PLT (×10⁹/microL)*</td>
</tr>
<tr>
<td>% of leukocytoreduction*</td>
</tr>
<tr>
<td>Time of procedure (min)</td>
</tr>
<tr>
<td>Volume of leukapheresis (mL)</td>
</tr>
</tbody>
</table>
| mean value and range in brackets.    |}

All patients signed a detailed informed consent. An immediate pre-apheresis blood count was carried out for every procedure; moreover, blood cell morphology was assessed by peripheral blood stream and May Grunwald staining. Leucocytes were removed by continuous flow centrifugation leukapheresis (COBE Spectra® device, Lakewood, CO, USA) utilizing citrate dextrose solution A as anticoagulant. The mononuclear cells (MNC) collection program was used in all cases, switching into manual mode and appropriately modifying the separation parameters. The separation factors of the device varied from 500 to 1000 depending on the target cells size (small lymphocytes or large blasts). The collect pump rate was always set at 5.0 mL/min. Patients were carefully monitored as respect to vital signs (blood pressure, heart rate, oxygen saturation). Calcium gluconate was infused i.v. continuously to prevent or minimize citrate toxicity. Isovolemia was maintained by carefully replacing the withdrawn volume with 5% human serum albumin in 0.9% NaCl solution i.v. continuously. In case of platelet count less than 20×10⁹/μL, prompt transfusion was administered before and/or after leukapheresis; red blood cell transfusion when necessary was delayed to completion of apheresis to avoid further increase in hyperviscosity. Results. From 2001 we have performed 34 apheresis procedures in 16 patients, whose characteristics are detailed in table 1 and 2. We obtained a decrease in circulating leukocytes (to less than 100×10⁹/μL) by a unique leukapheresis in 7 patients (44%) and by 2 procedures in 9 (56%). No significant adverse effects occurred. Conclusions. In our hands, the strategy based on the MNC program adapted for leukocytes morphology showed to be effective as well as highly tolerated and safe. This variant provided efficient leukocytes reduction; 5% albumin administration was able to preserve from the risks of hypotension even critically ill patients.

Therapeutic plasma exchange for thrombotic microangiopathy after hematopoietic stem cell transplantation: a single centre experience

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Thrombotic microangiopathy (TMA) after hematopoietic stem cell transplantation (HSCT) is an uncommon but serious complication. The etiology is still unclear although endothelial damage is a common mechanism. No effective treatment is available, although the therapeutic plasma exchange with fresh frozen plasma (FPP) could partially improve its manifestations. The aim of this study was to retrospectively analyse TMA cases in our Centre and their response to TPE. In the last 10 years, 389 patients underwent allogeneic HSCT, 10 of them (2.6%) developed TMA and initiated plasma treatment. These patients, 5 men and 5 women, with a median age of 50.5 years (range 22-72) were diagnosed with acute myeloid leukemia (AML) (n=4), acute lymphoblastic leukemia (n=2), paroxysmal nocturnal hemoglobinuria (n=2), non-Hodgkin’s lymphoma (n=1) and chronic myeloproliferative syndrome (n=1). It was the first allogeneic HSCT for 6 patients, the second for 3 and the third for 1 of them. The conditioning regimen was myeloablative in 5 patients and in the other separation parameters were as peripheral blood from HLA-identical related donor for 7 patients and the other one received bone marrow (1 HLA- identical related, 1 matched related, 1 identical unrelated). Cyclosporine (CSA) was used for immunosuppression.
graft-versus-host disease (GVHD) prophylaxis in 8 patients and Tacrolimus in the other 2. All patients had acute GVHD and 6 of them also developed chronic GVHD. Infection with cytomegalovirus was documented in 4 cases. The time of TMA onset after HSCT was 131 days (34-651). All patients had microangiopathic hemolytic anemia with 74 g/L (68-91) of hemoglobin, 4 scistocytes per field (2-10), a platelet count of 28x10^9/L (11-127), a reticulocyte percentage of 4.50 (0.18-6.55), lactate dehydrogenase (LDH) of 789 (574-174) and unconjugated bilirubin of 40 μmol/L (7.165). These patients also presented: renal insufficiency (n=7), neurologic abnormalities (n=6), coagulation abnormalities (n=6) and fever (n=2). When TMA was diagnosed, CSA was discontinued in all patients. Plasma infusion was assigned to 1 patient, TPE to 5 and 4 patients received the first regimen changed to TPE after 7 sessions (2-11). A total of 72 TPE, with a median of 8 (5-75) per patient, were performed using the Cobe Spectra cell separator. Those patients received an average of 2.5 L (1.4-3.9) of FFP with a fluids change of 100% remaining isovolemic. The procedures were well tolerated although the clinical improvement was poor. After TPE, renal insufficiency was still present in the 7 patients but headache had disappeared in all of them and LDH decreased to 518 U/L (277-6268). Since the diagnosis, the overall mortality was 90% after 32 days (4-203). The causes of death were multi-organ failure syndrome (n=7), infection (n=1) and progressive GVHD (n=1). In summary, TPE has shown inconsistent results. Even the patients who responded to TPE did not prolonged survival. However, early detection of TMA may allow advanced evaluation of the patient and change the disease prognosis.

1290 GLOBAL QUALITY OF LIFE OF PATIENTS WITH MULTIPLE MYELOMA AND MALIGNANT LYMPHOMA AFTER THE HSCT: A CROSS-SECTIONAL AND RETROSPECTIVE STUDY

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1University of Defence, HRADEC KRALOVE, Czech Republic; 2Psychiatric Clinic, HRADEC KRALOVE, Czech Republic; 3Department of Hematology, HRADEC KRALOVE, Czech Republic

Backgrounds. The cross-sectional and retrospective study analyses the selected factors which influence global quality of life (QoL) of patients with multiple myeloma and malignant lymphoma after the hematopoietic stem cell transplantation (HSCT). Aims. 1. to verify the applicability of the Czech version of an international generic European Quality of Life Questionnaire - Version EQ-5D for the evaluation of global QoL of patients after the HSCT at the Department of Clinical Hematology of the 2nd Internal Clinic in the University Hospital and Medical Faculty of Charles University in Hradec Kralove, Czech Republic, 2. to evaluate the global QoL of patients with multiple myeloma and malignant lymphoma (NHL) patients at the Department of Clinical Hematology of the 2nd Internal Clinic in the University Hospital and Medical Faculty of Charles University in Hradec Kralove, Czech Republic, 3. to analyse factors which influence global QoL of patients with multiple myeloma and malignant lymphoma after the HSCT at the Department of Clinical Hematology of the 2nd Internal Clinic in the University Hospital and Medical Faculty of Charles University in Hradec Kralove, Czech Republic; 4. to retrospectively evaluate patients' pain coping strategy. Patients and Methods. The total number of respondents after the transplantation from 2001 to 2008 was 80 and the return rate of questionnaires was 70% (56 respondents: 32 respondents (15 men, 14 women) with multiple myeloma and 24 respondents (11 men, 13 women) with malignant lymphoma). All respondents with multiple myeloma and malignant lymphoma were included in the analysis. The patients were selected for the study on the basis of their age (median age of 68 years (49-84)), sex, education, marital status, physical status, neurologic deficits and the type of disease (multiple myeloma (n=50); malignant lymphoma (n=30)). The results were statistically analyzed by means of dispersion analysis. Results. The average age of patients with multiple myeloma was 60 years and the average age of patients with malignant lymphoma was 44.5 years. The Czech version of an international generic Euro QoL Questionnaire - Version EQ-5D was used. The influence of monitored factors (age, sex, education, marital status, physical status, neurologic deficits, type of disease and the time lapse from the HSCT) on global QoL of patients was determined by means of dispersion analysis. Results. The above-mentioned factors proved statistically significant dependence of EQ-5D score and EQ-5D VAS on age (in both cases p<0.01), constant parameters in patients with multiple myeloma (in both cases p=0.05) and on type of disease (in both cases p=0.01). Conclusion. EQ-5D score and EQ-5D VAS significantly decrease with increasing age in both groups patients and with constant parameters in patients with multiple myeloma, and are significantly higher in patients with malignant lymphoma. The influence of other factors on EQ-5D score and EQ-5D VAS was not proven as statistically significant. The global QoL of patients with multiple myeloma after HSCT is lower (mean EQ-5D score was 68.9%, mean EQ-5D VAS was 66.6%) than in patients with malignant lymphoma after the HSCT (mean EQ-5D score was 82.7%, mean EQ-5D VAS was 76.7%).

1291 PAIN COPING MEASURING IN HEMOPHILIA: INDIVIDUAL VERSUS COMPOSITE SCORES

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Backgrounds. Coping with pain had been the object of studies for long time before the instruments for assessment in specific illnesses, like haemophilia, were constructed. Some research has already indicated that individual sub-scales in Cope Strategies Questionnaires (CSQ) are more meaningful than composites (Jensen MP et al., 1992). Aims. The aim of this study is to describe pain coping strategies among patients with haemophilia and find out if the results are related to the severity of the disease. Methods. A group of 24 adult patients with moderate and severe haemophilia is presented. The patients' coping with pain is assessed using the Pain CSQ Adapted for Haemophilia (PCSQ, Barry and Elander, 2002). This questionnaire was translated into Serbian, according to the recquired guidelines. Clinical severity of disease is measured using the frequency of bleeding episodes in the previous year (Solovieva S, 2001). Physical activity level is measured on a two-point scale. Statistical analysis, firstly performed, was based on the three originally defined factors in the PCSQ: negative thoughts about pain, coping attempts and passive adherence. Afterwards, it was based on 14 subscales, each one grounded on 5 to 6 items. Results. In the factor analysis, no differences are found in coping with pain between the groups with clinically severe and moderate disease (p>0,05), between patients with biologically severe and moderate haemophilia (p>0,05) and between those with difficulties in hard (moderate) physical activity and those with difficulties in any (no) activity (p>0,05). When using sub-scale scores, differences in pain coping strategies were found between the groups. Patients with difficulties in hard or moderate physical activities ignored pain sensations and increased behavioural activities, using them like preferred strategies more than people with difficulties in any or no activity (p<0,05). On the other hand, patients with difficulties in any activity used clotting factor and ice more often to cope with pain (p<0,05). Patients with clinically moderate disease also ignored pain sensations more willingly than those with severe haemophilia (p=0,026), who relied on praying and hoping (p=0,01) and used anger self-statements more when in pain (p=0,054). Patients with biologically moderate disease used ice more than those with severe disease (p=0,059). Summary. The results based on factor analysis suggest that the severity of hemophilia may not be the factor determining the type of patient’s pain coping strategy. The results based on sub-scales analysis suggest that possibly it would be better to analyze scores from the questionnaire in this way, rather than putting sub-scales together into three factors.

1292 FATIGUE IN NEW NON-HODGKIN’S LYMPHOMA PATIENTS, STRATIFIED ACCORDING TO THE INTERNATIONAL PROGNOSTIC INDEX

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Background. Fatigue is the most distressing symptom associated with cancer. Fatigue prevalence and severity observed during prior specific treatment may help to better understand the potential underlying mechanisms and to provide patient-centered treatment. At this time, the data on fatigue severity and its prevalence in new non-Hodgkin’s lymphoma (NHL) patients are lacking. The aim of this study was to describe fatigue prevalence and severity in new NHL patients, stratified according to the International Prognostic Index (IPI). Patients and Methods. 138 patients with newly diagnosed NHL – 76 aggressive (stage I-IV, mean age 60.0 SD=17.2, males/females 40/56) and 62 indolent (stage I-IV, mean age 59.8 SD=16.0, males/females 35/27) were included in this study. Each patient completed the Brief Fatigue Inventory (BFI) before treatment. To find out the relationship between fatigue and International Prognost-
tic Index we examined fatigue severity and fatigue interference within patient groups stratified by IPI. Results. Fatigue was reported by 77.3% of NHL patients predominantly in those with aggressive lymphoma (95%). Almost two thirds (60.5%) of patients experienced fatigue at the moderate-to-severe level. Aggressive NHL patients reported significantly more fatigue interference with patients’ daily lives than indolent NHL patients: mean RFI interference score 3.98 (SD = 2.50) vs 2.16 (SD = 2.55) (p = 0.05). The distribution of patients according to the IPI was as follows - IPI-1: IPI-2: IPI-3: IPI-4: 5.3: 5.3: 19.3: 70.1% for aggressive NHL and 40.0: 30.0: 20.9: 8.1% for indolent NHL. Fatigue severity differed significantly in the IPI groups (p = 0.001). Patients at low risk according to the IPI both in aggressive and indolent NHL groups had no fatigue. Patients at low-intermediate risk IPI experienced mild fatigue (mean 5.2, SD = 2.3 for aggressive NHL; mean 2.1, SD = 1.8 for indolent NHL). IPI-3 group was characterized by moderate fatigue (mean 5.7, SD = 1.5 for aggressive NHL; mean 4.1, SD = 2.4 for indolent NHL). Patients at high risk IPI had severe fatigue (mean 7.5, SD = 1.4 for aggressive NHL; mean 7.1, SD = 0.9 for indolent NHL). Significant differences in fatigue interference with patients’ daily lives were found across IPI groups (p = 0.001). Conclusion. Our results show that fatigue is a prevalent and distressful symptom in new NHL patients. It is much more pronounced in patients with aggressive lymphoma. Furthermore, we found that a certain IPI group is strongly distinguished by fatigue severity and its impact on quality of life. The findings support the suggestion that fatigue should be discussed as an important prognostic factor in this patient population.

1293

USAGE OF EICOSAPENTAENOIC ACID AND HIGH PROTEIN CONTAINING ENTERIC FEEDING PRODUCT IN MALIGNANCY RELATED WEIGHT LOSS OF CHILDREN

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The aims of nutrition therapy are preventing weight loss and improving functional capacity and life quality in cancer patients. Clinical effectiveness of standard nutritional support is limited in patients with tumor related weight loss. We aimed to observe weight changes of actively chemotherapy receiving patients by using eicosapentanoic acid and high protein containing enteral nutrition product (ProSure®) 46 patients [27 (58.7%) male, 19 (41.3%) female] were included to study. Mean age of patients was 80.5 ± 41.38 months (20-187 months). 39 patients had diagnosis of leukemia, 7 had of a solid tumor. All patients were receiving chemotherapy actively. Basal weight and body sizes of patients were recorded and they were suggested to use enteral products twice a day (morning - evening) in addition to their normal feeding. Patients were followed with regular intervals and their data were recorded. Their tolerance and regular use of the product were questioned. Patients were followed approximately 92±40.6 days. 33 (71.7%) patients had consumed and tolerated the product, 15 (28.3%) patients had consumed less than suggested amount because of its taste. Body weights of 20 (45.5%) patients were decreased while that of 9 (19.6%) were increased. No significant weight changes were observed in 17 (37%) patients. In conclusion, body weights of 80.4% of our patients were preserved, and that of 43.5% were increased.

1294

SATISFACTION WITH IRON CHELATION THERAPY IN PATIENTS WITH THALASSEMIA, SICKLE CELL DISEASE, AND MYELODYSPLASTIC SYNDROMES

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Backgrounds. Thalassemia, sickle cell disease (SCD), and myelodysplastic syndrome (MDS) patients require regular blood transfusions as part of their treatment programme. Regular blood transfusions can lead to iron overload (IO). Untreated, IO will result in morbidity and mortality. Removal of excess iron with currently available iron chelation therapy (ICT) requires 8-12 hour infusions, repeated 5-7 days per week. This time consuming therapy is burdensome to patients and may impact on satisfaction with ICT. Aims. To assess satisfaction with ICT and factors associated with satisfaction in patients with thalassemia (T), sickle cell disease (SCD), or MDS. Methods. A 28 item satisfaction questionnaire was developed and is currently being validated. The instrument comprises four domains that assessed satisfaction with ICT (acceptability; burden; perceived effectiveness; and side effects) and was administered at a single time point to 110 patients currently receiving ICT in the US (thalassemia: n = 41, SCD: n = 9) or UK (thalassemia: n = 40, SCD: n = 14, MDS: n = 6). Simple regression analyses were then conducted to explore factors explaining satisfaction with ICT. Results. The mean age was 30.87 years (SD = 14.95), with 63 females and 47 males. A total of 54% responded that they found their ICT inconvenient or very inconvenient compared to 26% who stated that they found their therapy convenient or very convenient. Further, 28% reported they were either very satisfied or satisfied with their prior ICT compared to 31% of patients stated that they were either dissatisfied or very dissatisfied. When asked ‘overall, how did the side effects of chelation therapy meet your expectations, 22% stated that they were either much better or somewhat better than their expectations, with 32% stating that they were either somewhat worse or ‘much worse’ than their expectations. Simple regression analyses revealed that whether patients experience side effects (R² = 0.15%, p < 0.0001), and the number of doses per week (R² = 7%, p = 0.007) were positively related to acceptance of ICT, whereas the number of doses missed in the last 7 days was negatively linked (R² = 6%, p < 0.01). Whether patients experienced any side effects (R² = 15%, p < 0.0001), patient’s disease (15%, p = 0.0008), and whether patients were unemployed (R² = 7%, p = 0.007), were significantly associated to satisfaction with burden of ICT. Whether side effects were experienced was also significantly and negatively associated with satisfaction with side effects (R² = 9%, p = 0.0001). Disease type (R² = 9%, p = 0.007) and unemployment status (R² = 6%, p = 0.009) were also significantly associated to satisfaction with side effects. Further, patients disease and patient’s place of residence explained 8% of the variance of perceived effectiveness (p = 0.02, p < 0.01, respectively). Summary/Conclusions. Results indicated that the majority of patients found their ICT inconvenient and one third of patients stated that they were dissatisfied with their ICT. Further, satisfaction is significantly influenced by a number of important factors related directly to ICT. Whether patients experience any side effects appears to be the single most important determinant of satisfaction and was associated with three of the four satisfaction domains: acceptability of ICT; burden of ICT and satisfaction with side effects.

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DOWN-REGULATION OF OSTEOCALCIN BY IMatinib-INDUCED INHIBITION OF CELL PROLIFERATION

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Backgrounds. Activated stem cells from haematological malignant tumours may show also characteristics of mesenchymal stem cells. In c-kit (CD117) positive cells, at least two splice variants of osteocalcin (OCN) were described. Aims. Aim of our study was to elucidate whether the expression of OCN and its splice variants relate to differentiation stages of haematopoietic cells (HL60) and an associated regulation of the transcription factors AML1 and AML3. Additionally, we wanted to clarify how cell-differentiating agents like vitamin D3 and imatinib (Glivec) affect proliferation of cells and expression of the genes named above. Methods. After incubation with differentiating agents (vitamin D3, imatinib), mRNA-expression of OCN, the transcription factors AML1 and AML3 and various metabolic genes were quantified by means of RT-PCR. Results. Our RT-PCR quantifications showed that after addition of vitamin D3 and imatinib, OCN appeared to be down-regulated. Alike observed at all marker genes for metabolism and haematopoiesis, the effect of inhibition of AML1 and AML3 was strongest with vitamin D3. After iatral nutrition, in all cell-lined analysed, the dose-dependent repression of proliferation is coupled with inhibition of OCN- and AML1-mRNA-expression. As opposed to the down-regulation of markers for immature cells, the differentiation marker Lox (lysyloxidase) was stimulated. Conclusion: In the cell-lines observed, differentiation leads to a decrease of the expression of OCN and the associated transcription factors. Further studies shall prove the effect of differentiation agents on healthy cells.
1296
CRYOPRESERVATION OF PERIPHERAL BLOOD PROGENITORS FOR AUTOLGOUUS TRANSPANTATION IN HEMATOLOGICAL MALIGNANCIES WITH DIFFERENT CONCENTRATION OF CRYOPROTECTANT - FIVE YEAR CENTER EXPERIENCE
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In this study we present our five year center experience with cryopreservation of PBSC and autologous transplantation in 42 patients with hematological malignancies treated in a period 2000-2005 at Department of Hematology, Skopje. Material and Methods. diagnosis of patients were (9 AML, 11 NHL, 10 MM, 12 HD) and median age at transplant was 34 years (7-63). Mobilization of PBSC was provided with Etoposide (VP-16) + G-CSF (10mcg/kg in AML patients, and high dose Cytosphamamide 4-5gr/m2)+G-CSF (10 mcg/kg or alone G-CSF) 10 mcg/kg in patients with lymphoproliferative diseases. Collected PBSC were cryopreserved in solutions with 5% DMSO in 20 patients and 10% DMSO in 22 patients, computer programmed until -80°C, and stored different period in liquide nitrogen on -196°C. Autologous transplant was performed with conditioning consisted of myeloablative high dose chemotherapy, BuCy in AML patients, high dose Mel in MM patients, BEAM in HD patients. CD34+ cell viability was assessed by fluorescence microscopy using acridine orange dye exclusion. Results. A total of 103 PBSC cryopreservation procedures were performed in our group of patients with median 3 (2-5) apheresis procedures. Median period from storage of cryopreserved PBSC grafts until thawing was 46 days (52-60). Total number of infused CD34+ cells was between 2.0-15×10^6/kg and median number of mononuclear cells was 4,2×10^6/kg(17-7,2). The amount of infused DMSO solution ranged between 210-650ml (median 480 ml) with DMSO concentration ranging 23 ml-50 ml (median 35 ml) in a group preserved with 10% DMSO and 18-28 ml (median 19 ml) in 5% DMSO cryopreserved grafts. The viability of the fresh harvests before storage was median 97% (range 65, 5-99, 9%). The poorer viability was associated with harvest cell count. Bellow 300x10^6/L the median viability was 98% and only 2/42 cases had ≤55% viable cells. Harvests count above 300x10^6/L the median viability was 78% (67,5%-99%). In a group of patients that received PBSC grafts preserved with 10% DMSO, also revealed signs of mild DMSO infusion related toxicity (22% vs14%). Hematopoetic recovery was similar in both groups, for Ne>0,5x10^12/L on day +9 (10,8-10) Pt>2x10^11/L on day +12 (11-14). Our results confirm that the infusion of cryopreserved autologous PBSC in hematological malignancies revealed successful engraftment in all patients and good cell viability. We did not registered hard to mobilize patients and graft failure.

1297
NO SIGNIFICANT INCREASE OF CIRCULATING CD34+ STEM CELLS IN PATIENTS AFTER ISCHEMIC STROKE
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Background. Stroke has a great socio-economic impact. Despite this the therapeutic influence on outcome is limited. There is an increasing suggestion that hematopoetic stem cells (HPC) may be able to induce repair processes after an ischemic event. Aim: Our objective was to determine whether patients with an ischemic cerebral event have elevated CD34+ stem cell number, leucocyte counts cells and granulocyte count. Methods. Ischemic stroke patients as well as time from stroke to admission seems to change. But subgroup analysis between cortical, subcortical and territorial stroke remained without a statistically significant change. But subgroup analysis of a larger patient group is necessary to elucidate a possible association between circulating CD34+ cells and stroke. However, the possibility that CD34+ cells home to the site of tissue damage without a measurable increase of circulating CD34+ cells still remains.

1298
ENHANCED ENGRAFTMENT OF HUMAN UMBILICAL CORD BLOOD DERIVED CD34+ STEM CELLS IN BALB/C MICE BY COTRANSPLANTATION OF MESENCHYMAL STEM CELLS
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Backgrounds. Umbilical cord blood (UCB) is considered as an attractive alternative source of hematopoetic stem cells for allogeneic stem cell transplantations. However the rate of UCB CD34+ stem cells graft is low. Mesenchymal stem cells (MSC) have been implicated in playing an important role in hematopoetic stem cell engraftment. Aims. In this study we examined the effect of human MSC on engraftment of human umbilical cord blood (UCB)-derived CD34+ cells in irradiated Balb/C mice. Methods. Human UCB CD34+ cells were obtained from full-term normal deliveries. Isolated CD34+ cells were counted and then cultured in Stemline II Hematopoietic stem cell expansion medium supplemented with 100 ng/ml SCF and 100 ng/ml TPO in 24-well plates. The cultures were incubated at 37°C in a fully humidified atmosphere with 5% CO2, and maintained over two weeks and half the medium was exchanged twice a week. Viability test was performed by trypan blue staining (100%). The direct determination of the absolute count of CD34+ was assessed by Flow cytometry (90%). Irradiated (7 Gy) Balb/C mice (n=20) were transplanted intravenously with 0.2 to 1.0×10^7 UCB CD34+ cells in the presence or absence of 0.25 to 1×10^6 culture-expanded human bone marrow-derived MSC. The mice in every group on day 11 after transplantation were killed and their spleen dissected. In every group colony assay were performed. For approving the presence of stem cells in colony, UCB CD34+ cells labeled with super paramagnetic iron oxide (SPIO) were transplanted. After establishing the presence of colonies in spleen, Prussian blue staining was performed. Results. Cotransplantation of low doses of UCB CD34+ cells (0.2 and 0.3×10^7) and MSC (0.5 and 1×10^7) resulted in a four-fold to five-fold increase in colony forming unit spleen, in comparison with engraftment of UCB CD34+ stem cells without MSC after 11 days. After Prussian blue staining Fe+ granules were observed. This indicates these cells in the colony were UCB CD34+ stem cells that were engrafted. Conclusions. The results showed that cotransplantation of MSC with UCB CD34+ cells promote engraftment of UCB CD34+ cells.
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EARY HEMOPOIETIC RECOVERY AFTER AUTOLOGOUS PERIPHERAL BLOOD STEM CELL (PBSC) TRANSPLANTATION IN RELATION TO THE NATURE OF THE INFUSED PRODUCT: UNSELECTED VERSUS SELECTED PBSC

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In the setting of autologous stem cell transplantation, purified CD34+ cell selection by immunomagnetic beads remove tumor cells from PBSC apheresis product, diminishing the relapse rate and outcome of treatment; some authors have reported that this purging may delay neutrophil and platelet count recoveries after transplantation. To analyze differences in early hematopoietic recovery (day in reaching neutrophil count > 0.5×10^9/L and platelet count > 20×10^9/L) after autologous PBSC transplantation, we selected versus unselected products are compared. We studied 160 consecutive autologous PBSC transplantsations (79 with unselected and 81 with selected PBSC), which were performed in our center over the last ten years. There were not statistically significant differences between both groups of patients in terms of infused cellularity. Unselected PBSC 3.36×10^9/kg and selected PBSC 3.35×10^9/kg (p=0.306). All patients received G-CSF daily (300 µg/kg, subcutaneous) from day 0 until the neutrophil count > 500/µm^3, for 3 consecutive days or Neutrophils count > 1000/mm^3. We did not find differences between the two groups in the day in which neutrophil early graft took place (unselected PBSC product, day +11, 11; selected PBSC product, day +11,11, p=0.104). Similarly, platelet recovery were not significantly different (unselected PBSC product, day +12,77; selected PBSC product, day +13,09, p=0.101). When analysis was performed based upon the infused cellularity, we found the following Results. 1) CD34+ cells infused < 2×10^9/kg: unselected PBSC product, day +12,17 for neutrophil recovery, vs day +12,50 for selected PBSC product, p=0.47; and unselected PBSC product, day +18 for platelets recovery vs day +19,83 for selected PBSC product (p=0.857). 2) CD34+ cells infused 2-4×10^9/kg: unselected PBSC product, day +11,12 for neutrophils recovery, vs day +11,57 for selected PBSC product (p=0.210) and unselected PBSC product, day +12,46 for platelets recovery vs day +15,11 for selected PBSC product (p=0.159). 3) CD34+ cells infused > 4×10^9/kg: unselected PBSC product, day +10,40 for neutrophils recovery, vs day +10,83 for selected PBSC product (p=0.757) and unselected PBSC product, day +11,87 for platelets recovery vs day +12,48 for selected PBSC product (p=0.287). In our study, we demonstrate that, although there is a tendency to a more delayed early graft for selected PBSC products, there were not statistically significant differences between selected and unselected autologous PBSC transplantations in terms of early hematopoietic recovery. This setting did not substantially vary when infused cellularity in each group was compared.

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EVALUATION OF MENISCOTAL STEM CELL EFFECT ON HOMING OF UMBILICAL CORD BLOOD STEM CELL IN BALB/C MOUSE WITH CLINICAL 1.5-T MRI

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Backgrounds. MSCs have been implicated as playing an important role in hematopoietic stem cell engraftment in co-transplantation experiments. We evaluated the effect of these cells in the homing of umbilical cord blood isolated CD34+ cells in irradiated and cyclosporine received mice by using clinical 1.5-T MRI. Aims. Making use of clinical 1.5-T MRI for tracking transplanted hUCB CD34+ cells in animal models and to evaluate the effect of MSCs in homing of these cells. Methods. Culture-expanded bone marrow derived MSCs were characterized by immune phenotyping and cultured under conditions promoting differentiation to osteoblasts or adipocytes. Culture-expanded umbilical cord blood-derived CD34+ cells were labeled with iron-oxide nanoparticles (Endorem™). Irradiated (7.5 Gy) and cyclosporine received Balb/c mice were transplanted intravenously with labeled UCB CD34+ cells in the presence or absence of culture-expanded MSCs. Mice underwent MR imaging with 1.5-T MRI equipment, before and after intravenous injection of UCB CD34+ cells labeled with SPION through simple incubation with protamine sulfate. Results. After injection of iron oxide-labeled hematopoietic cells, a significant decrease in MR signal intensity was observed in the bone marrow. The signal intensity reduction in bone marrow was significantly stronger after co-transplantation with MSCs, compared to transplantation of UCB CD34+ cells alone. Histochomical examination for Iron by the Prussian Blue Method in spleen colony forming units, confirmed these results. Conclusion: Co-transplantation of hMSCs with UCB CD34+ cells enhances their engraftment. This can be detected and evaluated in vivo with clinical 1.5-T MRI.

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HEPATIC VENO-OCCULSIVE DISEASE: A SINGLE CENTER EXPERIENCE WITH DEFIBROTIDE

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Backgrounds. Veno-occlusive disease (VOD) of the liver, renamed as sinusoidal obstruction syndrome is a common and possibly fatal complication of hematopoietic stem cell transplantation. The incidence of hepatic VOD being 10 - 60% varies according to diagnostic criteria and conditioning regimens. We retrospectively evaluated the laboratory values and daily progress forms of 65 consecutive cases who underwent hematopoietic stem cell transplantation. Patients and Methods. Sixtyfive patients with hematologic malignancies underwent HSCT and 68 trasplications were performed (31 autologous SCT, 37 allogeneic SCT with 3 of them being non-myeloablative SCT) between September 2003 to February 2006 with a median age of 42,5 years (range 16 - 71 years). Three patients received 2nd transplants, one as a part of tandem autologous protocol and two of them as retransplants after relaps of their disease. As a VOD prophylaxis, all patients received ursodexycolic acid, N-acetylcysteine and continous infusion of low dose heparin. The conditioning regimens consisted of cyclophosphamide/TBI, cyclophosphamide/busulfan and cyclophosphamide/busulfan/fludara-bine for the patients reported as VOD. VOD was clinically diagnosed with the development of two of the following features: hyperbilinurinemia > 2 mg/dl, hepatomegaly with right upper quadrant pain, and ascite or unexplained weight gain (> 5% increase of baseline body weight) within 30 days of transplantation. Patients were said to have multiorgan dysfunction if there was documentation of dysfunction of one other system in addition to liver dysfunction. The patients (16,2%) who were diagnosed as VOD were treated with defibrotide intravenously in doses ranging from 10 to 20 mg/kg per day for a median of 10 days (range 4 - 25 days). Serious adverse events due to defibrotide was not seen. At diagnosis median bilirubin was 4,6 mg/dl, median weight gain was 8,6%, ascite was present in 45,8% and hepatomegaly a right upper quadrant pain was present in 81,8% of patients. Severe VOD associated with multiorgan dysfunction was present in 2/11 patients (18,2%) with a 100% mortality rate before day 100. Severe VOD was reported to have a mortality rate approaching 100% by day +100 after transplantation which we also experienced in our 2 patients. In general 16/65 patients died (24,6%) and 6/65 (9,2%) of these deaths happened before day +100. Two out of six deaths (33%) happened before day +100 were due to VOD. Complete resolution of VOD was seen in 81,8% with a survival rate of 94,5% at day +100. Conclusion: Although there is still no satisfactory recommendation for the treatment of severe VOD, defibrotide seems to be the best therapy reported with acceptable side effects. In generally complete resolution of VOD was reported as 36 - 42% in the literature. The favorable complete response rate which we achieved in our series may be due to the early intervention of defibrotide therapy with the diagnosis of moderate to severe VOD in addition to the prophylaxis with ursodeoxylic acid, N-acetylcysteine and continous infusion of low dose heparin.

1302

CIRCULATING CFU-GM DURING HEMATOPOIETIC RECOVERY AFTER PERIPHERAL BLOOD TRANSPLANTATION: RELATIONSHIP TO GRANULOCYTE ENGRAFTMENT.

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Hematopoietic progenitor cells (HPC) are circulating in the peripheral blood (PB) before engraftment following auto or allo-PBSCT. It is known that CFU-GM constitutes a part of primitive progenitor cells. In the present study we investigated the kinetics of CFU-GM circulating in PB during the early stages following PBSCT. Patients: Forty seven auto and nine allo-PBSCT recipients were consecutively selected from January 2000 to August 2002 for this study. The median age was 46.5 years (17-69). 20 patients had non-Hodgkin’s lymphoma, 16 myeloma, 8 Hodgkin lymphoma, 7 acute leukaemia, 4 solid neoplasms and 1 patient chronic
myeloid leukemia. Cell preparation: 10 ml of PB in heparin was obtained on days 1, 4, 9, 11, 14, 16 y 18 after PBSCT. Mononuclear cells were isolated by Ficoll-Hypaque method. Progenitor assay: A standard methylcellulose colony assay (MethoCultTM GF H4531, StemCell Technologies, Vancouver, Canada) was used for analysing the number of CFU-GM on all of the days. Statistical analysis: Routines within SPSS (Statistical Package for the Social Sciences) were used for the estimates shown. CFU-GM decreased to undetectable on day 4 after transplantation. They appeared from day 9 to day 18 after transplantation, depending on the patient, along with neutrophil recovery. Figure 1 shows the post-transplant CFU-GM kinetics. We report on the detection of GFU-GM in 13 of 56 patients on day 9 and they number ranged from 2 to 10 per 10 ml PB depending on individual patients. On day 11 we detected CFU-GM in 43 patients, the number of them was 5-12. The number of CFU-GM on day 14 was 5-13 and they were detectable in 54 patients. On day 16 and 18 almost every patients showed CFU-GM colonies -55-, the number of them was 5-12. The number of CFU-GM plant CFU-GM kinetics. We report on the detection of GFU-GM in 13 patients, along with neutrophil recovery. Figure 1 shown the post transplant kinetics. GFU-GM decreased to undetectable on day 4 after transplantation. They reappeared from day 9 to day 18 after transplantation, depending on the patient, along with neutrophil recovery.

Figure 1. Kinetics of CFU-GM in PB after PBSCT.

1303 SIDE EFFECT OF STEM CELL MOBILIZATION WITH GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF) ON MORPHOLOGY AND FUNCTION OF LIVER IN MICE

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Mobilization of hematopoietic stem and progenitor cells (HSPC) from the bone marrow into circulation can be induced in patients and animal models by a wide variety of molecules including hematopoietic cytokines, such as recombinant human granulocyte colony-stimulating factor (rhG-CSF). A cytokine-mobilized HSPC may ‘home’ to peripheral hematopoietic organs as well as to other sites, for example to liver. However, the morpho-physiology of liver during this process and the safety of G-CSF mobilization are poorly appreciated. To address this problem, the morphology and function of liver in healthy mice after short treatment with G-CSF were investigated. Female (CBAX C57Bl/6)F1 mice 16-20 week-old of age were used. The mobilization of HPSC was induced with 200 mg/kg of rhG-CSF injected daily for 8 days. The mice were killed 1, 7 and 28 days after last injection of G-CSF. Peripheral blood was collected and white blood cells were counted. Cell morphology was evaluated on May-Grünwald-Gimsa-stained blood smears. The liver function was monitored by serum bilirubin, enzyme alanin- and aspartat aminotransferase (ALT and AST) levels. Formalin-fixed, paraffin-embedded liver sections were stained with haematoxylineosin and Picro-Sirius red for histological examination of livers. Beginning at day 1 after last injection of G-CSF till the end of experiment (day 28) the number of peripheral blood leukocytes did not change and was the same as in control mice. Differential analyses performed on blood smears revealed that the number of mature neutrophils increased significantly after treatment of mice with G-CSF, reaching maximal values on the day 7 after ablation of cytokine. Liver in mice receiving G-CSF revealed classic liver lobules with normal architecture and with only mild hepatocellular necrosis. However, on day 7 after the last injection of G-CSF numerous erythrocytes were observed in the lumina of the central and lobular veins. Some erythrocytes and hemosiderin-containning macrophages and the foci of granulopoiesis were visible outside the vessels mainly around the portal area. Deposition of erythrocytes accompanied with slight bilirubin and AST elevations. At day 28 after mobilization most of the vessels were empty but liver sinusoids were distinctly dilated. Unexpectedly, it was observed that almost all blood vessels walls were reinforced and symptoms of fibrosis were visible. Liver vessels walls composed of a single layer of endothelial cells and no more than two layers were visible. Picro-Sirius red-positive staining revealed the presence of collagen synthesis since day 7 (all vessels) till day 28 (mainly portal tract vessels) after mobilization. Distribution and density/friability of the collagen fibers network was more prominent at the end of experiment. A strong correlation between liver morphology and microsomal enzyme induction was not demonstrated. G-CSF treatment causes morphological, but no functional changes in murine liver. The side effects observed were associated with extramedullary granulopoiesis in liver and with liver blood vessels thrombosis. These adverse effects were partly reversible at 4 weeks post-mobilization. G-CSF stimulates indirectly liver stromal cells to produce collagen, however, time-dependent collagen degradation was not observed in this set of experiment. A long-term follow up is required.

1304 THE EFFECTIVENESS OF PREOPERATIVE ERYTHROPOIETIN IN PEDIATRIC SURGERY

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Backgrounds. The idea about bloodless surgery because of patients’ personal or religious concerns, especially in the pediatric population, is challenging. Concerns about the transmission of the human immunodeficiency virus (HIV) have driven the evolution of surgical transfusion practices including the use of preoperative erythropoietin (EPO). Despite the fact that the consequences of transfusion-related diseases are very important issue for pediatric population only a few studies have examined the potential benefits of preoperative erythropoietin in children. Aims. The aim of this study is to assess the clinical efficiency of EPO as preoperative treatment in two children scheduled for heart and abdominal surgery without blood transfusion. Methods and Results. Two children are presented in this study: 15 and 3 year-old boys Jehovah’s Witness. They underwent a major surgical procedure without blood transfusion (surgery of the choledochal cyst and corrective treatment of Fallot’s tetralogy). Patient that is 15 years old suffered an abdominal pain and presented right upper abdominal mass in association with jaundice eight months before. He underwent clinical, laboratories examinations and ultrasonography. The diagnosis of the choledochal cyst was delivered. Surgery was made at the Department of Pediatric surgery, Medical Faculty in Skopje. The second patient, 3 years old, had discrete microanomaly and fatigue. Diagnosis of Fallot’s tetralogy was established by clinical, laboratory examinations, ECG and Echocardiography. A successful surgery procedure was performed at the Pediatric cardiology, Deutsches Herzzentrum Berlin. Both operations were carried out without the use of blood products through the application of multidisciplinary effort. Preoperative EPO treatment was administered subcutaneously with 300 U/kg weekly for four weeks (three weeks before surgery and one after surgery). In addition, patients received an oral iron, folic acid and vitamin C supplementation. The effectiveness of preoperative EPO treatment was followed through the RBC count, hemoglobin concentration, hematocrit, platelets, and hematocrit. Stimulation of erythropoiesis was seen with an increase of the reticulocyte count (42 and 61‰) by day 3 of the treatment. The increase of the hematocrit (first patient: from 39.2 to 44‰; second patient: from 40.1 to 43.6‰), hemoglobin concentration (first patient: from 13.7 to 15.1: second patient: from 12.3 to 14.7g/dl) and increase of RBC count were registered after 3 weeks of treatment with EPO. Both patients didn’t receive blood transfusion. EPO caused no adverse reactions. Conclusions. Preoperative EPO treatment has been shown to be an effective alternative to red-cell transfusion in children undergoing surgery. A significant hematopoietic response with no side
effects was achieved in this study. With respect of this study, but also in accordance with data available from literature, preparative EPO may be used more often in pediatric surgery.

1305 EVALUATION OF IL-1β, IL-2, IL-4 AND TGF-α IN PATIENTS WITH THE MALIGNANT HEMATOLOGICAL PATHOLOGY
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Interleukines (IL) are the main homeostasis regulating agents and they have very wide spectrum of different biologic effects. They take part in regulation of all components of immune system and execute local immune answer in malignant process but the question about how interleukines realizing their enormous possibilities for anti-tumor-resistance activation and why these possibilities may be diminished in organism with growing tumor mainly are unclear now. Optimization of prognostic criteria and efficiency of treatment for patients with different malignant hematological pathology on the basis of IL-1-β, IL-2, IL-4 and TGF-α monitoring, 39 patients (3-61 years) with different malignant pathology (acute leukemia -22 (recipients of autoPBSCT) (ALL)-8 and children (LL)4; HD-8; MM - 9 were investigated. 25 pts received autoPBSCT. For patients undergoing autoPBSCT the investigation carried out at the following points: before conditioning treatment, after autoPBSCT in time of restoration of hemopoiesis, through +3 - +17 months after transplantation. For children with ALL underwent only for conventional chemotherapy, investigation executed in the acute period and in the remission. The evaluation of spontaneous IL-1-β, IL-2, IL-4, TGF-α production in supernatants of daily cultures of peripheral blood mononuclear cells were measured by ELISA (Diaclone, France). The control group consisted of 22 healthy persons. Very low IL-1β levels were revealed in all patients groups before auto PBSCT, its level fluctuated within 13-65 pg/ml limits. In the early post-transplant period IL-1β level raised up to 162,28±38,95 pg/ml only in patients with HD (p<0,05), meanwhile in AL group IL-1β level continue to come down (range 7,97-13,47 pg/ml) and in MM group was stable low. IL-2, IL-4 levels in PBSCT recipients group did not differ reliable from those in control in all points of observation except HD. There were higher IL-2, IL-4 in these patients before transplantation (79,11±48,5 pg/ml and 3,29±1,21 vs 13,62±0,7 2 pg/ml and 1,25±0,52 pg/ml in reference group respectively, p<0,05), which became almost normal after PBSCT (20,07±5,77 pg/ml and 1,40±0,58 pg/ml (p<0,05)). TGF-α/α levels were low in HD before transplantation and in ALL relapse groups (61,05±17,74 pg/ml and 85,4±25,50 pg/ml vs reference 141,94±101,01 pg/ml). In HD early post-transplant period and in ALL remission group TGF-α/α level raised up to normal value (401,27±111,34 pg/ml and 520,25±123,04 pg/ml, respectively, p<0,05). The obtained data indicate the preliminary activation of patient’s immuno-competent cells in vivo. The different differences in IL-1-β, IL-2, IL-4, TGF-α/α levels in view of disease, its course, treatment with PBSCT and outcome were revealed. The presented data reflects the implications of inflammatory cytokines (TGF-α/α, IL-1β) in the pathogenesis of ALL in children and HD as treatment effectiveness with autoPBSCT implant. We conclude that investigation of these cytokines production may be helpful in optimization of prognostic criteria and treatment effectiveness of the specified pathology.

1306 ERYTHROPOIETIN SIGNALING IN PANCREATIC TUMOR CELL, AR42J: ACTIVATION OF MITOGEN ACTIVATED PROTEIN KINASES AND THEIR EFFECT UPON CELL PROLIFERATION
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Erythropoietin (EPO) regulates the proliferation and differentiation of erythroid progenitors via its receptor, EPO-R and through various Mitogen activated protein kinase (MAPK) pathways. We reported last year at 10th EHA Congress that high dose EPO could enhance the proliferation of rat pancreatic tumor cell line, AR42J. Aim: To extend this study to examine the activation of two MAPKs, namely, extracellular regulated kinase 1/2 (ERK1/2) and c-jun NHE-terminal kinase 1/2 (JNK1/2) in AR42J. Methods: AR42J cells were cultured, exposed to 5 mU/mL of EPO and cell extracts were prepared. These were separated by electrophoresis and subjected to Western blot analysis. EPO induced proliferation was evaluated by 5'-Bromo-2'-deoxyuridine (BrdU) incorporation method. We found a rap-

id activation of ERK1-2/2 in AR42J cells reaching the maximum of 8.3 fold in 5 min after EPO exposure, while it took 30 min for JNK1/2 to reach the maximum. To examine the effect of induction of MAPK by EPO on AR42J cell proliferation, cells were treated with inhibitors to ERK1/2 and JNK1/2, PD98059 and SP600125, respectively, for 1 hour prior to EPO addition and the cell proliferation were measured from day 1 through 4. EPO addition to AR42J cell culture resulted in significantly higher proliferation on day 2 and it was 1.93±0.09 absorption units compared to the value of 0.47±0.05 absorption units seen in controls without EPO at that interval (p<0.01). When cells were treated with ERK1/2 inhibitor prior to the addition of EPO, proliferation was significantly suppressed to 0.53±0.05 absorption units at that interval (p<0.01). Similarly, with JNK1/2 inhibitor and EPO a significantly decreased cell proliferation (0.43±0.06 absorption units, p<0.01) was observed. These results indicate that in AR42J cells, for EPO mediated proliferation, activation of both ERK1/2 and JNK1/2 are necessary and indicates a role of MAPK in EPO induced proliferation of tumor cells. This aspect has to be taken into account for any treatment involving EPO.

1307 EXPRESSION OF LYMPHOID T-MARKERS ON GRANULOCYTES AFTER GM-CSF AND G-CSF ADMINISTRATION
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Background: Granulocyte-macrophage colony stimulating factor (GM-CSF) and granulocyte colony stimulating factor (G-CSF) affects the function and phenotypic expression of granulocytes by different mechanisms that include: (a) direct activation of granulocytes; (b) indirect activation due to the production of other cytokines (such as interleukin-6, and tumor necrosis factor-α); (c) increased mobilization rate from the bone marrow, and therefore granulocytes still express immature markers in peripheral blood; and (d) G-CSF acts on granulocyte precursors and reduces the period of maturation in bone marrow. The effect of GM-CSF and G-CSF on granulocytes function and phenotype is complicated and depends on the dose, the route and the way (bolus, continuous) of growth factor administration. There are also differences between in vitro and in vivo effects. Aim: The aim of this study was to evaluate the effect of GM-CSF and G-CSF on granulocyte phenotypic expression. Methods: We determined the phenotypic expression of granulocytes obtained from the peripheral blood of 26 patients with hematological malignancies (7 patients had AML, 7 ALL, 4 CML, 5 non-Hodgkin’s Lymphomas, and 3 MDS) and 7 patients with solid tumors. Blood samples were collected on the 1st and 2nd day of growth factor administration, as well as on days 5-20 after the final day of growth factor injection. Phenotypic analysis was performed by the alkaline phosphatase (AAPAP) immuno-cytochemical technique, using a wide panel of monoclonal antibodies. Results, Granulocytes expressed T antigens on their surface after GM-CSF or G-CSF administration as depicted in table 1. High percentage of granulocytes expressed CD4 on their surface, in most cases after the 5th day from the beginning of G-CSF administration. In addition, they expressed transiently CD7 and CD2 at the same time or later in relation to CD4 expression. Conclusions: CD2, CD4 and CD7 are adhesion molecules and they act as activation molecules. CD4 and CD7 are also markers of immaturity. The expression of those antigens on granulocytes indicates: (a) premature differentiation of granulocytes resulting in preservation and expression of immature markers on their surface; and (b) activation of granulocytes. The in vivo administration of GM-CSF and G-CSF induces the expression of T antigens on granulocytes during activation of the antigen-presenting system.

Table 1. Percentage of CD2+, CD4+ and CD7+ granulocytes.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 5</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>0.30</td>
<td>0.5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>ALL</td>
<td>0.75</td>
<td>0.50</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>CML</td>
<td>0.30</td>
<td>0.20</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>MDS</td>
<td>0.65</td>
<td>0</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>NHL</td>
<td>0.80</td>
<td>0.35</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>Solids</td>
<td>0.50</td>
<td>0.65</td>
<td>0.70</td>
<td></td>
</tr>
</tbody>
</table>
Backgrounds. G-CSF (granulocyte colony stimulating factor—eg Filgrastim) regulates the production of neutrophils within the bone marrow and also impacts on their function. It is frequently given to patients with leucopenia or neutropenia caused by various underlying diseases. The treatment with G-CSF is apparently safe although flares in patients with autoimmune diseases and cutaneous vasculitis have been described in the literature. Selected patients with myelodysplasia (MDS) with suppurative infections may be a candidate for G-CSF although there is no data to support their routine use in MDS. Other common side effects of G-CSF are musculoskeletal pain, transient hypotension, deranged LFTs, thrombocytopenia, dysuria, proteinuria, haematuna, allergic reactions and transient decrease in blood glucose. Splenic enlargement, hepatomegaly, headache, diarrhoea, epistaxis, alopecia, osteoporosis and reactions at injection site have been reported.

Figure 1. Rash following G-CSF/histology of rash.

Case history: We report a 55 yr old man who presented to the A/E with a 2 week history of feeling generally unwell with decreased appetite, muscular aches and pains (all joints) and nausea. He had background history of Type II Diabetes Mellitus and hypercholesterolaemia. Blood tests on admission revealed that he had neutropaenia (N=0.6) mild anaemia (Hb =12) mild thrombocytopenia and hyponatraemia (Na 123). His bone marrow raised the possibility of early myelodysplasia although his karyotype was normal. An ultrasound scan of the abdomen revealed mild splenomegaly of 14.4 cm. A CT scan revealed a pulmonary nodule 1x2 cm. A lung neoplasia as aetiology of his hyponatremia was queried. However a PET scan was negative. He presented to the A/E two weeks later with febrile neutropaenia and was treated with intravenous broad spectrum and G-CSF subcutaneously the following day. About 7 days later he developed a non itchy widespread maculopapular rash over his scalp, trunk (front and back) arms (distally) and legs. G-CSF was discontinued. A skin biopsy was consistent with leucocytoclasia. Further investigations revealed: ANA positive in 1/400; dsDNA positive in High titre; complement titres—low. SLE seemed like—ly diagnosis and he was commenced on Prednisolone (1mg/kg). His WCC was 2.58 and Neutrophil count was 1.54.

Conclusion: G-CSF is often prescribed very freely in leucopaenia caused by multisystem disorders. In this case it caused a severe widespread rash which was very worrisome for the patient and the family. Hence before considering G-CSF for unexplained leucopaenia an autoimmune screen should be checked along with a bone marrow aspirate/biopsy. G-CSF should be avoided in untreated Lupus and other vasculitides(if possible) in view of risk of flare up.
and 40 μmol/L quercetin respectively, there were marked down-regulation of MRPI gene expression, as compared with mock-treated group (p<0.01). In HL-60 cell line, the DNR fluorescence was mainly distributed in the nucleus, cytoplasm and cell membrane, with nucleus intensely, cytoplasm uniformly and diffusely, membrane continuously staining pattern (Figure A). Compared with mock-treated group, the distributions of DNR fluorescence were not obviously changed after treated with different concentrations of quercetin (Figure B). However, in HL-60/ADR resistant cells, DNR fluorescence was mainly distributed in periphery region of cytoplasm and membrane, the granule was not homogeneous, and fluorescence signal was hardly seen in the nucleus (Figure C). Nevertheless, as concentration of quercetin increased, fluorescence signal was gradually increased in the nucleus and cytoplasm. When the concentration of quercetin increased up to 40 μmol/L, the fluorescence intensity almost reached level of that in sensitive cells with diffuse granule distribution (Fig. D). Altered subcellular distribution of DNR in resistant cell line was related to MDR gene formation in tumor cells. Quercetin could inhibit MRPI function and restore the subcellular distribution of DNR in vitro.

1311
RETINOIC ACID AFFECTS THE RESPONSE OF V-MYB-TRANSFORMED MONOBLASTS TO OKADAIC ACID

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Background. Okadaic acid (OA) inhibits serine/threonine protein phosphatases 1 (PP1) and 2A (PP2A), thus inducing differentiation and/or apoptosis of various leukemic cell lines in dose-dependent manner. This suggests that PP1 and PP2A phosphatases actively participate in regulation of these processes. Moreover, retinoic acid (RA) affects expression and activity of the PP2A. Aims: The aim of this study was to explore the functional interactions of RA- and OA-driven pathways in v-myb-transformed monoblasts BM2. We have previously described that BM2 monoblasts ectopically expressing Jun, RA-receptor (RAR) or retinoid X receptor (RXR) proteins differentiate to macrophage-like cells upon treatment with RA while wild-type BM2 cells do not respond to RA. Results: In this study we found that 10 nM OA induces adherency, cell cycle arrest, phagocytic activity, production of reactive oxygen species and expression of vimentin in BM2 cells. These features that mark differentiation along monocyte/macrophage pathway are enhanced in BM2 cells upon simultaneous treatment with OA and RA. Interestingly, the 20nM OA induced apoptosis is greater than differentiation of BM2 cells as documented by analysis of cell morphology, chromatid condensation, internucleosomal DNA fragmentation and fosfmatidylserine translocation. This proapoptotic effect of OA in BM2 cells was inhibited by RA. Conclusions: These results indicate that pro-differentiation and pro-apoptotic effects of OA on BM2 monoblasts are differently regulated by RA.

Funding: This work was supported by the grant 304/03/D022 of the Grant Agency of the Czech Republic and by the grant MSM0021622445 of the Ministry of Education, Youth and Sports of the Czech Republic.

1312
STUDIES OF PNAS-2, AN ANTI-APOTOPSIS GENE

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We use gene chip and find PNAS-2 (Gene ID:AF229832) is one of the down-regulated genes upon treatment of As454 in APL cell line-NB4, same result has been reported by other group except the configuration of arsenic sulhide is As2S3 instead of As4S5. Moreover, PNAS-2 expression unchanged in U937 and K562 leukemia cell lines. The hypothetic protein of PNAS-2 shows high sequence similarity to a protein that is thought to be involved in apoptosis, however, there are no studies characterizing this gene. To obtain 5' unknown sequence of PNAS-2, PNAS-2-GFP-fusion proteins express plasmid was constructed, after transfected to U937 cell line, Western blot analysis was applied to detect GFP fusion proteins; Northern Blot was used to detect the expression of PNAS-2 gene in the multi-tissue; Real-Time PCR was applied to detect PNAS-2 expression in patients. After RT-PCR, we found two splice patterns of PNAS-2 in NB4 cell lines, as F1 PNAS-2 and F2 PNAS-2; both were more than 95% homology to CHMP5, CGI-34 and HSPC177, these genes had a same open reading frame (Figure 1). After transfected GFP fusion protein expression plasmid to U937 cells, we applied Western blot analysis. The results confirmed PNAS-2 could be translated into protein and it was not a pseudogene (Figure 2). Northern Blot was applied in the multi-tissue including heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, lymph node, thymus, leukocyte, bone marrow and fetal liver, we found no expression of PNAS-2 gene in majority tissues except in placenta (Figure 3, 4). After Real-Time PCR, we found PNAS-2 expression increased and activity of higher in a case of acute leukemia (AL) include 71 de novo and 6 relapse when compare with 8 complete remission (CR) patients (p=0.0001) or 57 non-tumorous disease patients (p=0.0003). (Figure 5). There was no statistic difference between each subtype of AL.
The deletion of the long arm of chromosome 20, hereafter del(20q), is a common cytogenetic abnormality in various myeloid disorders and is known to be a favorable prognostic factor in myelodysplastic syndromes (MDS). However, del(20q) is sometimes found to be associated with disease progression and is detectable as one of additional cytogenetic changes. In order to ascertain the risk factors in MDS, we analyze 33 patients with MDS showing del(20q). We categorized del(20q) into two types; one is the sole and major del(20q) clone (>50% marrow metaphases) corresponding to genomic integrity; while the other is a late appearance of minor del(20q) clone (<50% metaphases) with additional cytogenetic changes representing genomic instability. Of the MDS patients with del(20q) at initial presentation, the negative factors in predicting prognosis on survival are (1) more progressive disease status, (2) any additional cytogenetic changes, or (3) minor del(20q) clone. CONCLUSION: The late appearance of del(20q) at any phase is linked to a significantly unfavorable prognosis, thus indicating the clinical and biological heterogeneity of del(20q) in MDS.

**11th Congress of the European Hematology Association**

**1314 REVISIT OF DEL(20Q) IN MYELODYSPLASTIC SYNDROMES (MDS): RISK FACTOR ANALYSIS IN MDS PATIENTS WITH DEL(20Q)**


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The deletion of the long arm of chromosome 20, hereafter del(20q), is a common cytogenetic abnormality in various myeloid disorders and is known to be a favorable prognostic factor in myelodysplastic syndromes (MDS). However, del(20q) is sometimes found to be associated with disease progression and is detectable as one of additional cytogenetic changes. AIMS: In order to ascertain the risk factors in MDS, we analyze 33 patients with MDS showing del(20q). We categorized del(20q) into two types; one is the sole and major del(20q) clone (>50% marrow metaphases) corresponding to genomic integrity; while the other is a late appearance of minor del(20q) clone (<50% metaphases) with additional cytogenetic changes representing genomic instability. Of the MDS patients with del(20q) at initial presentation, the negative factors in predicting prognosis on survival are (1) more progressive disease status, (2) any additional cytogenetic changes, or (3) minor del(20q) clone. CONCLUSION: The late appearance of del(20q) at any phase is linked to a significantly unfavorable prognosis, thus indicating the clinical and biological heterogeneity of del(20q) in MDS.

**1315 THE 5’KIAA1509/3’PDGFRB FUSION GENE IN MYELOPROLIFERATIVE DISORDERS**

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**Background.** The myeloproliferative disorders (MPDs) are characterized by the abnormal proliferation of one or more myeloid cell types. Unlike the Philadelphia chromosome in chronic myeloid leukemia, there is no specific chromosomal abnormality associated with the MPDs. However, a number of recurrent chromosomal rearrangements, involving a variety of tyrosine kinase genes as PDGFRα, PDGFRβ, FGFR1, and JAK2, have been reported. In this report, we describe a patient with MPD bearing a t(5;14)(q31.2;q32). As a consequence of this rearrangement, the 5′ region of the KIAA1509 gene was fused to the 3′ portion of PDGFRβ. Aims. We performed a molecular cytogenetic analysis by FISH to identify the genes mapping in correspondence to chromosomal breakpoints. Further molecular studies have been carried out to exactly define the breakpoints location within KIAA1509 and PDGFRβ genes. The presence of this chromosomal translocation was investigated by fluorescence in situ hybridization (FISH) analysis on additional MPD cases. Methods. FISH experiments were performed with BAC clones specific for KIAA1509 and PDGFRβ genes. The presentation of this chromosomal translocation was investigated by fluorescence in situ hybridization (FISH) analysis on additional MPD cases. Results. FISH experiments were performed with BAC clones specific for KIAA1509 and PDGFRβ genes. The 5′ KIAA1509/3′ PDGFRβ fusion transcript was detected using a KIAA1509 exon 11 forward primer (KIAA1509-11F) and a PDGFRβ exon 11 reverse primer (PDGFRβ-11R). The fusion protein domains were identified using the BLAST program (http://www.ncbi.nlm.nih.gov/blast). A FISH screening of 12 MPD cases was carried out. Results. FISH cohybridization experiments with RP11-356B9 and RP11-754F9 probes specific for KIAA1509 and PDGFRβ genes revealed their involvement in the reciprocal translocation. RT-PCR analysis with KIAA1509-11F and PDGFRβ-11R primers produced an amplification product of about 200 bp. The sequence analysis demonstrated that breakpoints were located within KIAA1509 intron 11 and PDGFRβ intron 10. According to molecular data, the fusion protein was composed of 2 N-terminal KIAA1509 domains (coiled-coil myosin heavy chain tail and chromosome segregation ATPase region)

We found PNAS-2 highly expressed in 71 de novo AL patients compared with 8 CR patients (p = 0.0001); PNAS-2 also highly expressed in 6 relapse AL patients compared with CR patients (p=0.0166), but there was no statistical difference between de novo AL patients and relapse AL patients (p=0.0759)(Figure 6). We also found PNAS-2 expression notice-ably decreased in 6 AL patients when achieved CR significantly compared with CR patients.

Figure 1. After blasted on website, the result showed both F1 PNAS-2 and F2 PNAS-2 were more than 98% homology to CHMPS, CGI-34 and HSPC177. Not only the ATG initial code of protein translation but also the open reading frame were completely same in these genes, indicated F1 PNAS-2, F2 PNAS-2, CHMPS, CGI-34 and HSPC177 were alias of the same gene as them had been presumed and they could be translated to same protein. Figure 2. Western blot analysis Result: M:marker; PNAS-2: cells transfected with PNAS-2-pcDNA3.1/CT-GFP-TOPO vector showed a GFP-fusion protein was detected and it was about 30-40KD; con1 was transfected with Control 1-pcDNA3.1/CT-GFP-TOPO vector, a GFP-fusion protein about 20-30 KD had been detected; con2 was U937 cells which had been transfected with Control 2-pcDNA3.1/CT-GFP-TOPO vector and could observe a GFP-fusion protein about 20-30 KD; con3 was cells transfected with Control 3-pcDNA3.1/CT-GFP-TOPO vector, and could not find fusion protein. According to our design, the cells transfected with PNAS-2-pcDNA3.1/CT-GFP-TOPO vectors could express a PNAS-2-GFP fusion protein which is about 34 KD; transfected with Control 1-pcDNA3.1/CT-GFP-TOPO vectors could express a fusion protein consist of three histones, connective region peptide and GFP which is about 28KD; transfected with Control 2-pcDNA3.1/CT-GFP-TOPO vector only can express a 26 KD GFP protein and U937 cells transfected with Control 3-pcDNA3.1/CT-GFP-TOPO vectors can express neither GFP protein nor connective region peptide. This result coincided with our design, our findings confirmed PNAS-2 could be translated into protein and it was not a pseudo-gene. Figure 3, 4. MTN hybridization Result: 1 heart; 2 brain; 3 placenta; 4 lung; 5 liver; 6 skeletal muscle; 7 kidney; 8 pancreas; 9 spleen; 10 lymph node; 11 thymus; 12 leukocyte; 13 bone marrow, 14 fetal liver. PNAS-2 mRNA expressed only in placenta. Figure 5. Grouped t test showed PNAS-2 expression statistically higher in 77 acute leukemia patient de novo and non-tumorous disease patients. Figure 6. Analyse mean DCT of PNAS-2 expression change and course of disease in AL patients: high expression at onset stage, decrease when achieve CR and increase again at relapse, it seems PNAS-2 gene may contribute to leukemogenesis.
and a C-terminal PDGFRB domain (catalytic tyrosine kinase). The use of different primers combinations revealed the absence of the reciprocal 5’ PDGFRB/3’ KIAA1509 fusion transcript. The FISH screening of 12 MPD patients with BAC clones specific for KIAA1509 and PDGFRB genes did not reveal the presence of other cases bearing the 5’ KIAA1509/3’ PDGFRB fusion gene. The patient with 5’ KIAA1509/3’ PDGFRB fusion transcript, was treated with imatinib and achieved hematological remission; the molecular response is still under evaluation. Conclusions. In this study we report the second MPD case with a t(5;14)(q32;q32) bearing a 5’ KIAA1509/3’ PDGFRB fusion gene. Our case differ from that previously reported in literature as KIAA1509 breakpoint was mapped within intron 11 instead of intron 9; any difference was observed in PDGFRB breakpoint position. As a consequence of this diversity, in our case a larger fusion protein was produced including an additional chromosome segregation ATPas domain. Treatment with imatinib resulted in hematological response in both cases. Our data illustrate how molecular cytogenetic techniques may be useful to uncover recurrent chromosomal rearrangements in MPD patients.

1316 DETECTION AND MONITORING OF CYTOMEGALOVIRUS(CMV) IN BONE MARROW TRANSPLANT (BMT) RECIPIENTS BY REAL-TIME PCR (RQ-PCR)

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CMV has been recognized as the most important viral pathogen in persons undergoing BMT. Monitoring of CMV reaction from latency is critical for these patients. We could detect CMV DNA in this patients by RQ-PCR For monitoring of CMV reaction. If copy number of CMV was increased, preemptive therapy will be initiated. 51 recipients of BMT (9-51 years) were monitored as weekly intervals until day 100 after transplantation. For amplification of the pp65 gene (UL83) RQ-PCR assay and pp65 Antigenemia method were preformed in parallel with 815 samples. By cloning of this region, we made standards for RQ-PCR. The results obtained by the two techniques were significantly correlated (p<0.01). We could detect 15x106-15x107 copies/2x109 cells by RQ-PCR. 76% of patients developed more than one episode of CMV replication. First positive result of RQ-PCR 13 days earlier than the Antigenemia. After preemptive therapy 16 days (7-21 days) needed to become negative result of RQ-PCR. There was no relationship between death and increase of CMV copy(p<0.419). There is no correlation between copy number of CMV virus and PP65 and WBC count (p<0.824, p<0.422). ROC-Predictive that pp65 Antigenemia. After preemptive therapy, negative results of RQ-PCR were the best indicator for determining of successful treatment. Reaction of CMV in our patients mostly endogenous and depend on kind of immunosuppressive therapy. If copy number of CMV increased one log, CMV reaction developed 1.2 fold.

1317 MONITORING OF MINIMAL RESIDUAL DISEASE AND TREATMENT OF MOLECULAR RELAPSES IN PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA

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Backgrounds. Minimal residual disease monitoring of PML/RARA chimeric transcript is widely used method for detecting of molecular relapses (MR) in pts with APL during hematological remission. However, we need the necessity of therapy changing when MR is detected is still debated. Aims. We tried to find out whether the PML/RARA detection during hematological remission ultimately leads to relapse of APL and to develop the optimal treatment strategy of MR in APL pts. Materials and Methods. We investigated bone marrow samples by RT-PCR for PML/RARA chimeric transcript in 73 pts with newly diagnosed and morphologically proved APL. Primers synthesis for nested RT-PCR was performed using recommendations of BIOMED-1 Concerted Action (1999). RT-PCR was performed on fresh marrow aspirates of all pts before treatment and periodically (2-Monthly) during all period of therapy (2 years after induction of remission). MR was defined as probable if chimeric transcript was detected once and was not found out by second investigation and as proved when PML/RARA was detected at least twice by consecutive investigations (in 2-4 weeks). Results. In 69 pts (94.5%) PML/RARA chimeric transcript was revealed during first investigation. 31 (45%) demonstrated bcr1 type of transcript, 33 (55%) - bcr2 type. In 4 pts (5.5%) PML/RARA was not found. During maintenance therapy in 19 of 52 pts (36.5%) MR was detected. In 5 patients from 6 with proved MR and in 3 pts from 13 with probable MR therapy was changed for Ara-C with idarubicine in early MR (12 months from remission induction) or ATRA + Interferon alfa in late onset of MR. No one of these pts developed hematological relapse. Maintenance was not changed in 11 pts (10 with probable MR, one - with proved MR) and 4 (36%) of them subsequently relapsed (one with proved MR).Conclusions. According to our data, detecting of PML/RARA in pts during maintenance therapy leads to high incidence of relapse in APL pts. Changing of therapy during MR significantly decreases the probability of hematological relapse from 36% to 0% (p=0.001).

1318 MONOCONAL ANTIBODY TO CD34 INHIBITS PROLIFERATION AND INDUCES APOPTOSIS OF CD34+ STEM AND MEYOLOYD CELL LINES

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Backgrounds. Monoclonal antibodies to epitopes of membrane differentiation antigens are widely used for therapeutic targeting of tumor cells. Aims. In some hematological malignancies, however, there is a need for more specific antibodies targeting the surface epitopes expressed on immature hematopoietic cells. Methods. We developed mouse monoclonal antibody of IgG1 class, clone 4H11, reactive with the common (protein) epitope of human CD34 molecule. We detected the antiproliferative effect of CD34 antibody on human CD34+ stem and myeloid cell lines. Inhibition of proliferation was tested by uptake of triitated thymidine and apoptosis was detected by Annexin-V-Fluoroscente kit. Results. Anti-CD34 antibody 4H11 inhibited proliferation and induced apoptosis of CD34+ positive cell lines at the concentration between 1-200ug/ml after 12, 48 and 72 hours. The anti-CD34 antibody strongly inhibited proliferation and induced apoptosis of all CD34+ cell lines (MOLM-9, JURL, HEL, RPMI 8402) but not control CD34 negative cells. The antiproliferative effect was detected even at the antibody level of 2.5ug/ml, and the antiproliferative effect was potentiated by simultaneous presence of differentiation inducing cytokines. The expression of CD34 antigen at the surface membrane of tested living cells was not modulated by 4H11 antibody. Conclusions. Based on the results obtained by the ex vivo model system of cultured leukemia cells we suggest that antigenic epitopes expressed on CD34 molecule should be considered as possible new molecular targets for the development of more effective targeted therapy of severe hematological malignancies, especially of immature myeloid lineage. (Supported by grant NR/8233-3 of the Internal grant agency of the Ministry of Health of the Czech Republic).

1319 ESTIMATION OF THE DIAGNOSTIC VALUE OF MYELOPEROXIDASE INDEX AND LDH IN MEGALOBLASTIC ANEMIA

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Most cases of megaloblastic anemias corresponded to anemia with hyper-segmented neutrophils, macroovalocytosis and very high serum LDH level. Elevated neutrophil myeloperoxidase index (MPXI) may be indicative of a diagnosis of megaloblastic anemia. The aim of this study was to estimate the value of MPXI and LDH in the diagnosis of macrolblastic anemia to facilitate the diagnostic algorithm prior to performing any bone marrow aspirate. MPXI and LDH were assessed using the first blood sample obtained prior to any transfusion or medical therapy, and after therapy in 29 patients diagnosed as megaloblastic anemia. MPXI was assessed using complete blood count (CBC), performed by Techni-con H1 (Bayer) instrument. Mean value of MPXI significantly decreased after treatment (20.4, C195%: 17-23 vs. -0.75, C195%: -4.27, before and after treatment, respectively). The same significant pattern was also observed for LDH (4250, C195%: 3906-5369 vs. 783, C195%: 492-1075, before and after treatment, respectively). The proportional diagnostic value (%) was significantly higher when both MPXI and LDH (53 percent, p<0.001) were used together in the diagnosis of Megaloblastic Anemia while the same index was (71 percent, p<0.001) for MPXI and (48 percent, p<0.001) for LDH when they were used alone. MPXI and LDH values may have a diagnostic role on megaloblastic anemia. It might be used as a reliable screening tool before doing any other diagnostic procedure.


**1320 INTERFERENCE OF HBA1C DETERMINATION BY THE HEMOGLOBIN VARIANT SHELBY**

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Heterozygosity for Hb Shelby was serendipitously discovered in an asymptomatic 48-year-old African American male with a normal complete blood count following ion-exchange high-performance liquid chromatography (HPLC) determination of glycated hemoglobin (Bio-Rad Variant A1c). Aims. Characterization of the Shelby hemoglobin variant and consideration of its interference with HbA1c assessment. The patient had a recent history of blood glucose levels in the normal range (average value = 90 mg/dL), inconsistent with the measured HbA1c level of 12.9% (normal range 4.8-6.2%). Of note, the interpretative software used for the A1c analysis identified the patient as having sickle cell trait (Hb A/S). Re-assessment of the degree of glycation using a boronate affinity column gave a more clinically appropriate value of 8.9% (normal range 4.0-6.0%). Additional HPLC analysis using the β-Thal Short Program (Bio-Rad) displayed an unknown peak comprising 26.3% of the total signal with a retention time of 4.84 minutes. Two previously described α-globin variants with similar retention times, O-Indonesia and O-Arab, displayed peaks with distinct conformational differences (slender peak bases versus a broad peak base) and associated glycation products not observed in our patient. Liquid chromatography-mass spectrometry (LC-MS) on a Finnigan LCQ using electrospray ionization showed a β-globin peak with a molecular weight of 15868 amu and a 738 amu shift from the α-globin peak, isobaric with the normal β-globin peak; all three exons of the β-globin gene were sequenced bidirectionally at ARUP Laboratories. Heterozygosity for a nucleic acid mutation CAG→AAG at codon 131 (conferring a GLN→LYS amino acid substitution with zero mass change on MS), consistent with Hb Shelby, was found. This hemoglobin variant is described as unstable. Although asymptomatic, the patient did show a striking increase in the number of target cells on a peripheral smear. This report emphasizes the need to correlate laboratory findings with associated clinical parameters and to question results that do not appear reasonable. Determination of glycated hemoglobin by boronate affinity HPLC has been demonstrated as helpful in the monitoring of blood glucose control in patients with hemoglobinopathies.

**1321 PREVALENCE OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN THE MALE POPULATION OF THE IRANIAN PROVINCE OF HORM OzGAN**

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Backgrounds. G6PD deficiency or Favism is a red cell enzymopathy, which is very frequent in certain areas of the Persian Gulf and in African and Mediterranean countries. Aims. A relatively high incidence of G6PD deficiency and neonatal jaundice carried us to study the prevalence of G6PD deficiency in the Hormozgan province, an area in which about 1.2 million people live. Methods. We randomly selected 816 male individuals aged between 15-20 years living in Bandar Abbas, Haji-Abad, Bandar Lengeh, Roos, Minab, Jask and Geshem Island. The G6PD activity in the red cells was measured and classified in five categories, from (I) the (lowest) to V (the highest enzyme activity) and anemia level, according to WHO recommendations. Results. Our survey showed a heterogeneous variety of G6PD phenotypes: 3 cases (0.36%) in class I, 118 cases (14.46%) in class II, 134 cases (16.42%) in class III, 506 cases (62.69%) in class IV and 1 case (0.1%) in class V. The average hemoglobin levels in class I was 10.2 ±0.6 gm/dL and in other classes was within normal ranges. The geographical distribution of the prevalent rates was as below: class I (1.8%) and class II (10.5%) in the Geshem Island, Class II (23.9%) in the Haji-Abad, and class IV (77.1%) in the Bandar Lengeh areas. Our study showed that the mildest clinical symptoms (class IV) were found in the Bandar Lengeh area. The amount of NADP substrate needed to reach half of maximum reaction velocity (KM) was 9.1±1.75 (μmol/L) for class II, 3.8±0.9 (μmol/L) for class III and 3.6±1.8 μmol/L for class IV while the Km G6P for class II, III, and IV were 31.1±12.8, 44.8±11.2, and 44.8±11.2 μmol/L, respectively. Conclusion. Hematological indices and clinical parameters were normal in heterozygote carriers, who showed mild chronic anemia, the rest did not present any significant clinical symptom. However, some individuals had transient jaundice episodes in childhood. Hematological indices where also measured and a high percentage of the studied individuals (57%) presented with Hb A/S more than 80. This is probably due to the high prevalence of α- and β-thalassemia traits and possibly iron deficiency in the areas. Conclusion. This study indicates that diagnosis and classification of G6PD deficiency should be routinely included in the public health care in the Hormozgan province. Moreover, further investigation is required for a better characterization of this disease at the molecular level.

**1322 SEVERE IGA MEDIATED AUTOIMMUNE HEMOLYTIC ANAEMIA IN HODGKIN’S LYMPHOMA PATIENTS: CASE REPORT**

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Backgrounds. In very rare cases a severe IgA-mediated autoimmune haemolytic anaemia may be observed in Hodgkin lymphoma patients. Here is reported such a case. Case report. A 21-year-old man presented an anaemia with a dyspnea but without fever, loss of weight, pain, sweat, adenopathy, hepatomegaly nor splenomegaly - Two years before, a Hodgkin lymphoma with thoracic adenopathy and paraeriticus had been diagnosed (Nodular sclerosis, stage II B). A chemotherapy including 6 AVBD cycles and a radiotherapy (30 + 10 Gy) had been performed and a remission had followed - At day (D) 0, an anaemia (Hemoglobin 87 g/L, hematocrit 25.8%, and red blood cell (RBC) count 2.4 tera/L) was observed but without leucopenia nor thrombocytopenia. A hyperbilirubinemia (48 μmol/L) was associated with a sharp drop in the haptoglobin level (lower than 0.2 g/L). There was a high rate of lactocdedehydrogenase (LDH) (1007 IU/L), but transaminases, C-reactive protein and fibrinogen levels were normal. A hemolytic anemia was suspected. Two D later, the anaemia was unchanged and the hemolysis confirmed, but the aetiology was not established. At D8, as the anemia was getting worse (Hemoglobin 75 g/L), new tests were carried out and a cortico-therapy quickly started. The clinical course became satisfactory and no other immunosuppressive therapy or RBC transfusion were needed. Moreover, no allo- and auto-immune RBC antibodies were screened and identified by indirect (IAT) and direct antiglobulin test (DAT). The tests were performed using gel cards. In the DAT, anti-human IgG, IgM, IgA, C3c and C3d Ab were used. For the IgA auto-Ab testing, a second DAT with another anti-human IgA Ab was carried out by gel and tube methods. Results : For the first sample at D2 the IAT was negative and with the second gel DAT, a negative result was observed with the IgG and -C3d Ab. Whereas on the second sample at D8, the gel DAT performed was negative with anti-human IgG, IgM, C3c and C3d Ab but strongly positive with anti-IgA Ab. Using the second anti-IgA Ab, a strong positive reaction was also obtained in gel test, but negative in tube. Another gel test was carried out on the D2 sample but with anti-IgG Ab ; results were similar to those of the D8 sample. Summary/conclusion: IgA-mediated autoimmune haemolytic anaemia is rarely observed in Hodgkin lymphoma patients. When results are negative in the DAT with anti-human IgG, IgM, C3c and C3d Ab and the aetiology not established, a DAT with anti-IgA Ab is then recommended to detect these IgA RBC auto-Ab.

**1323 α-THALASSEMAIA CARRIERS IN CRETE: HEMATOLOGICAL AND MOLECULAR STUDY**

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Backgrounds. α-thalassemia is probably the most common monogenic disease. The prevalence of this disorder in different populations varies greatly ranging from less than 1% to over 90%. In the Greek population α-thalassemia appears to be quite heterogenic with frequency of carriers up to 8.3%, a percentage similar to that of α-thalassemia. Aims. To present the hematological and molecular findings of 69 α-thalassemia carriers in Crete, a region of Greece with increased incidence of α-thalassemia. Methods. Erythrocyte parameters and erythrocyte membrane and haemoglobin F were measured in 69 α-thalassemia carriers who were born in Crete and had no history of sickle cell disease. A confirmatory test for α-thalassemia was carried out with High Performance Liquid Chromatography (HPLC), while methyl-violet dye after incubation was used for the detection of HbH inclusions. Serum iron and ferritin concentrations were
determined with colorimetric and immunoenzymatic techniques, respectively. GAP PCR, and hybridization with allele oligonucleotides (ASO) were used for the molecular analysis for the most common α-thalassemic defects found in the Greek population, the deletional defects - αα1, ααβ, α2β, and the non-deletional defects IVS1-5, pentanucleotide deletion, PolyA (AATAAA→AATAGC) and (AATAAA→AATGGA), Hb Argentina and others. Statistical analysis was carried out with the Student's t-test. Results. Among the 72 α-thalassemic chromosomes of the 59 αα and 10 αβ-thalassemia carriers, 44 (61.11%) deletional and 28 (38.89%) non-deletional chromosomes were found. The deletions in the deletional chromosomes were the -αα in the S7 chromosomes (84,09%), the ααβ in 6 chromosomes (13,64%) and the ααβ in one chromosome (2,27%). The molecular defects in the non deletional chromo-
somes were the IVS1-5, pentanucleotide deletion in 23 chromosomes (62,14%), the PolyA TSaudi mutation in 3 chromosomes (10,72%) and the Hb Icaria mutation in 2 chromosomes (7,14%). All the non deletion-
al defects were related to the β-globin gene. Among the α-thalassemia carriers, MCV and MCH values were lower in IVS1-5, pentanucleotide deletion carriers than in -αα deletion carriers (p<0.001 and p<0.001 respectively). No statistically significant differences were noted among the other erythrocrit parameters of these carriers. Summary/Conclusions. A higher percentage of non-deletional chromosomes, a higher percent-
age of the IVS1-5, pentanucleotide deletion and a lower percentage of the PolyA TSaudi mutation were observed in the α-thalassemia carriers in Crete compared to the previously reported percentages found in the general Greek population.

1324
APOLIPOPROTEIN E GENOTYPES IN IRANIANS WITH SICKLE CELL DISEASE

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Background. Cardiac abnormality is one of complications in most of patients with sickle cell disease. Apolipoprotein E plays an important role in lipid metabolism. The apoEε4 allele has been known to be associated with risk of myocardial infarction and coronary artery disease. Aims. To determine the genotypes of apolipoprotein E and the frequency of apoE genotypes in children with sickle cell disease. Patients and Methods. Patients studied included 35 sickle cell anemia of which 21 were males and 14 females (age 8-41 years), 15 sickle/β-thalassemia patients, 8 males and 7 females (age 6-46 years) and 15 sickle cell trait individu-
als, 9 males and 6 females (age 1-58 years). Sickle cell phenotype was determined by microcolumn chromatography method. DNA was extracted from whole blood using phenol-chloroform procedure. Apo E genotypes were analysed using PCR followed by digestion with Hha I restriction enzyme. Results. Of the six possible apo E genotypes, four were observed in sickle cell anemia patients that were ε3/ε3 (65.7%), ε3/ε4 (17.1%), ε2/ε3 (14.3%) and ε2/ε2 (2.9%). The frequencies of apo E alleles were: ε3 (81.4%), ε4 (10.0%) and ε2 (8.6%). In sickle cell trait individuals the order of frequencies of apo E genotypes was: ε3/ε3 (80.0%), ε3/ε4 (6.7%) and ε2/ε2 (13.5%). In sickle/β-thalassemia patients only two apo E genotypes (ε3/ε3, 36.7% and ε2/ε3, 63.3%) were exist-
ed. Summary/conclusion. It was concluded that the high frequency of apoE ε4 allele in Iranians with sickle cell anemia might increases the morbidity and mortality results from cardiac abnormalities in these patients.

1325
MOLECULAR CHARACTERIZATION OF Glucose-6-phosphate DEHYDROGENASE DEFICIENCY IN WESTERN IRAN

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Background. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a worldwide enzymopathy affecting an estimated 400 million people. Its presentation generally occurred as hemolytic episodes after ingestion of fava beans, unripe peaches, drug administration or infections. In many populations, the molecular defects responsible for this disease are one or a small group of mutations present at high frequency. Aims. To study the spectrum and frequency of G6PD mutations in school boys of West-
ern Iran. Methods. The studied subjects were 64 G6PD deficient individ-
uals comprised of 52 school boys aging 14-18 years diagnosed during schools screening and 12 children aging 1.5-13 years with history of fav-
ism and acute hemolytic anemia. All individuals were Kurds from Kermanshah province. DNA was extracted from whole blood by the phe-
nol-chloroform method. Detection of mutations in coding region of G6PD gene was performed using PCR-RFLP analysis for the characteri-
zation of the G6PD Mediterranean and PCR-SSCP technique for the screening of exons 2 through 13. All mutation detected by SSCP were confirmed by an ABI system. Results. The G6PD Mediterranean muta-
tion (563 C>T) was detected in 57 males and one female, who was heterozygous for this mutation giving an allele frequency of 90.62%. G6PD Chatham (1003 G:A) in exon 9 was found in 5 males (7.81%). Nucleotide (nt) sequencing of exon 12 revealed a G:C substitution at nt 1376 (G6PD Cosenza) in one subject (1.56%). All but three individuals with G6PD Mediterranean mutation were of Mediterranean ethnicity. Nevertheless the strong association of the G6PD Mediterranean mutation in Kurds from Western Iran is higher than those from two Fars ethnic groups living in Northern and Southern Iran. Nevertheless they are in stricty accordance with previous report of the prevalence of the G6PD Mediterranean in Kurdish and Middle East population. Also, the strong association of the G6PD Mediterranean mutation and the presence of the polymorphism nt-1311 C>T in the Kermanshah population demonstrate, that the presence of this mutation may be the result of migrations that have taken place through the history.

1326
ASSESSING ERYTHROPOIESIS IN HEMODIALYSIS PATIENTS; THE IMPACT OF PRO-HEPCIDIN LEVELS

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Background and Aims. Prohepcidin (pro-HPC) is the precursor of hep-
cidin (HPC), a liver-derived peptide involved in iron metabolism by blocking its intestinal absorption and its release by the reticulo-endотhe-

lial system. Iron overload and inflammation increase HPC expression, whereas anemia and hypoxia suppress it. In the present study pro-HPC levels were determined in the serum of hemodialysis (HD) patients and its correlations with iron metabolism markers, C-reactive protein (CRP) and hematocrit (Hct) were assessed. Patients and Methods. 46 HD patients (M/F: 24/22, mean age: 61.1±12.8 years, mean time on HD: 52±24.8±5 months) and 22 healthy volunteers (M/F: 11/11, mean age: 52.2±19.2 years) were studied. Hct, serum pro-HPC, CRP, iron, ferritin, transferrin saturation and soluble transferrin receptors (sTFRs) were measured. Weekly erythropoietin dose and last month intravenous iron dose were recorded. Results. In comparison to healthy volunteers, HD patients had higher serum ferritin (539.77±96.87 vs. 67.82±48.27 ng/ml, p<0.001), sTFRs (0.465±0.173 vs. 0.307±0.109 mg/dl, p<0.001) and CRP (0.904±1.044 vs. 0.186±0.115 mg/dl, p<0.001), lower serum iron (64.45±35.3 vs. 99.77±41.62 ng/dl, p<0.001), Hct (84.1±3.14 vs. 86.53±5.7%, p<0.001) and similar transferrin saturation (22.83±13.33 vs. 21.81±11.59%, p ns) and pro-HPC levels (257.46±96.87 ng/ml vs. 234.00±130.82ng/ml, p ns). In the patients' group pro-HPC levels were negatively correlated with Hct (p: 0.022) but not with any of the other examined parameters. Multiple linear regression analysis considering age, inflammation, iron adequacy, erythropoietin dose and prohepcidin levels revealed that prohepcidin was the predominant determinant of Hct (p: 0.06). Conclusions. Taking into account the low Hct levels in HD patients of our study, it seems plausible that the pro-HPC levels assessed in this group are inappropriate high. These functionally high pro-HPC levels may belong to the factors that inhibit erythropoiesis in HD patients. On the other hand, the absence of other expected correlations indicates that further studies are needed in order to definitely clarify this aspect.

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ROLE OF ERYTHROPOIETIN IN THE TREATMENT OF PATIENTS WITH SEVERE HEART FAILURE

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Pathogenesis of anaemia in congestive heart failure (CHF) is multifac-
torial. Although the biological mechanisms linking anaemia and heart failure are not completely understood, prevalent anaemia is consistent
in patients with severe heart failure and is associated with higher mor-
tality rates. Erythropoietin (EPO) promotes erythrocyte survival and dif-
ferentiation and develops multiple paracrine-autocrine functions that
cordinate local responses to injury. We investigated the effect of EPO
administration in patients suffering from heart failure compatible with
New York Heart Association functional classes III to IV. Twenty-four anae-
mic patients, 14 males, median age 62 years (range 47–77) were
studied and received EPO (15000 IU per week) for three
months. The rest of them comprised the control group. Heart failure
functional class was comparable in both groups. Therapy included treat-
ment with digoxin, angiotensin-converting enzyme inhibitors or All
blockers, carvedilol and diuretics and was not different between the
groups. There was no well tolerated by all patients. They underwent echocar-
diography in order to evaluate systolic and global left ventricular func-
tion. Ejection fraction (EF) and Tei index (calculated by dividing the sum
of isovolumetric contraction and relaxation time by ejection time) were
estimated at baseline and at the end of the study. Hemoglobin, creati-
nine and electrolytes were measured at baseline and every month later.
Significant increase in hemoglobin values (10.2±0.5 g/dL to 14.2±0.7
g/dL, p<0.01) were observed in the EPO group, but no significant
changes in the control group. Echocardiography showed improvement
in left ventricular systolic and global function in the EPO group (EF
42±5% vs 48±6%, p<0.01, Tei index 0.58±0.14 vs 0.42±0.09, p<0.01),
while echocardiographic indices remained unchangeable in the control
0.05; OR = 2.589). While they were elevated in 677T/T (but not C/C)
mRNA analysis revealed in two
patients with the thalassemia trait. 880 patients (396 men and 484 women)
in left ventricular systolic and global function in the EPO group (EF
42±5% vs 48±6%, p<0.01, Tei index 0.58±0.14 vs 0.42±0.09, p<0.01),
while echocardiographic indices remained unchangeable in the control
group. 2/3 patients of the control group were hospitalized due to decom-
pression of heart failure and none in the EPO group. A slight decrease
in creatinine values in the EPO group was detected at the end of the
study, probably indicating improvement in renal vessels flow, but it was
not statistically significant. EPO significantly improves systolic and glob-
al LV function, cardiac capacity and decrease of hospitalizations.
Normalization of Hb concentration in patients with CHF may interrupt a vicious cycle, the recently coined cardiac-renal-anemia
syndrome. EPO may have a direct positive effect on the heart unre-
lated to correction of anaemia. Possible mechanisms could be preven-
tion of tissue damage by reducing cell apoptosis and increasing neovas-
cularization.

1328
THE IMPACT OF BONE FORMATION MARKER-OSTEOCALCIN-IN PATIENTS WITH β-TALASSEMIA
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The life expectancy of patients with thalassemia has greatly improved
over the last years as a result of regular transfusions and increased com-
pliance with iron chelation therapy; however, this improvement is often
accompanied by a series of serious complications including osteopenia
and osteoporosis. The pathogenesis of these skeletal disorders is multifac-tion-
al, due to the increase in bone resorption and increased bone formation via
desferoxamine toxicity. The non invasive assessment of bone turnover has markedly improved with the development of specific and sensitive
markers of bone formation. The aim of this work is to assess the value of
some bone formation markers in patients with β-thalassemia. To
achieve this goal 36 thalassemia patients were recruited in this study, they
were 20 males (56.6%) and 16 females (44.4%) and their ages ranged from 3-18 years, beside 20 apparently healthy subjects of
matched age and sex serving as a control group. The patients were select-
ed from outpatient clinic and inpatient of Hematology/oncology Unit
of Mansoura University Children’s Hospital (MUCH). The selected sub-
jects were subjected to thorough history taking, clinical examination,
radiological evaluation and laboratory investigations including: com-
plete haemogram, serum iron, serum ferritin, TIBC, serum calcium,
phosphorus and estimation of bone formation markers as alkaline phos-
phatase and osteocalcin. The results revealed that serum calcium level
was within the normal range and showed no statistical significance. The
differences were detected in 81 heterozygous patients (numbers of patients
with a particular mutation are in square brackets): IVS1-6(T→C) [32];
IVS2-745(C→G) [28]; IVS2-1-G→A) [11]; IVS1-1-G→A) [4]; CD6’ A [2];
CD6’ T [4]; IVS1-1-G→A) [5]. DNA analysis revealed in two patients
(of two unrelated families) an internal mutation IVS1-6(T→C) in homoyzote stage. Frequencies of individual muta-
tions in Poland were different from those encountered in Mediterranean and Some Central European countries.

1330
CONTRIBUTION OF MTHFR C677T AND A1298C SINGLE NUCLEOTIDE POLYMORPHISMS
TO THE GENETIC SUSCEPTIBILITY OF SICKLE CELL DISEASE
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Backgrounds. Methyltetrahydrofolate reductase (MTHFR) catalyzes
the homocysteine-to-methionine conversion, and a reduction in its activity
leads to elevation in homocysteine (Hcy) levels (hyperhomocystein-
e mia), a recognized risk factor for several thrombotic events. The MTHF-
FR single nucleotide polymorphism (SNPs) C677T results in thermola-
bile enzyme and induces hyperhomocysteinemia, more than the
A1298C SNP. Insofar as sickle cell anemia (SCA) is associated with a
hypercoagulable state, many candidate genes were proposed to induce a
prothrombotic state in SCA patients, including the MTHFR C677T
SNP. Aims. This study addressed the prevalence of C677T and A1298C
MTHFR SNPs among Bahraini SCA patients and control subjects, and
correlate the genotype with changes in Hcy levels. Method: this was a
case control study. Study subjects comprised 106 SCA patients (68 male
and 38 female; mean age 15±9.8) and 165 healthy controls (80 male
and 79 female; mean age 27±15.1); all were Bahraini nationals. Muta-
tions analysis was assessed by PCR-RFLP analysis using Hinf I (C677T)
and Mbo II (A1298C). Statistical analysis was performed on SPSS v. 13.0
statistics software. Fisher’s exact test and Pearson’s χ2 test were used to
assess inter-group significance, set at p < 0.05. Results. The frequencies
of C677T and A1298C alleles of C677T and A1298C were comparable
between patients and controls. Higher frequencies of the C/C variant of
the A1298C but not C677T TT (p=0.67) SNP were seen in patients than in
controls (p<0.03; RR = 2.55). Differences between patients and con-
trols in C677T and A1298C distribution were also noted in haplotype
distributions. The elevated C677T and A1298C haplotype was detected in patient
(p=0.05; OR = 2.589). While they were elevated in 677T/T (but not C/C)
carrier, Hcy levels were comparable between patients and controls. Sum-

11th Congress of the European Hematology Association

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mary/conclusion: Results from this study showed that A139C, but not C677T SNP, was associated with SCD. While the mechanism underlying C/C effect was not addressed here, it’s not likely to involve changes in Hcy levels, since Hcy level was comparable between patients and controls.

**1331**

**VITAMIN B12 DEFICIENCY AND THROMBOSIS**

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Backgrounds. Hemoglobinopathies (HbP’s) are the most prevalent genetic disorders. Acquired hyperhomocysteinemia may cause thrombosis in vitamin B12 deficiency. Aims: To evaluate the thromboembolic events in patients with vitamin B12 deficiency. Methods: One hundred forty-three patients (64 female, mean age 59±8 years) with vitamin B12 deficiency (vitamin B12 level < 200 pg/ml) were enrolled to this study. In a control group, there were 129 healthy persons (62 female, mean age 58±8 years). Upper gastrointestinal endoscopy was performed to 102 patients. Antibody to parietal cells and the levels of homocysteine were examined in 78 and 36 patients, respectively. In last three years, arterial and venous thromboembolic events were detected. χ2 and student t-test were used in the comparison of two groups. Results. Thromboembolic events were detected in 9.8% of the of patients vitamin B12 deficiency. The sites of thromboembolic events were coronary arteries in 9 patients, deep venous and cerebrovascular thrombosis in two patients, respectively. There were thromboembolic events in 3.9% of controls. These rates were not different in two groups (p>0.05). The levels of homocysteine were high (> 20 μmol/L) in all of 36 patients. Conclusion. Thrombosis was not higher in vitamin B12 deficiency. Although we did not examine the levels of homocysteine in all of the patients, hyperhomocysteinemia may not contribute to thrombosis. More extended studies should be done in this topic.

**1332**

**VALIDATION OF A DEDICATED HPLC METHODOLOGY FOR THE IMPLEMENTATION OF NEONATAL SCREENING OF HEMOGLOBINOPATHIES. A TOOL FOR PRIMARY AND SECONDARY PREVENTION IN A MULTI ETHNIC SOCIETY**

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Backgrounds. Hemoglobinopathies (HbP’s) are the most prevalent recessive disorder in Man. At least 300.000 affected children are born each year from parents who are both healthy carriers, most of them in endemic countries. Migrations have drastically changed the composition of the populations and non-endemic countries where the HbP’s are growing. The number of patients in The Netherlands will double in the next decade if no prevention is offered. Although only partially effective for primary prevention the Ministry of Health recently decided to include the screening for hemoglobinopathies, and of sickle cell disease in particular, in the existing neonatal screening program. Aims. We have validated the Variant Newborn Blood Screening (VnbS) HPLC apparatus (Bio-Rad) to determine if and how it could be used at a maximum of diagnostic efficiency in a national screening program for HbP. We intended to study how neonatal screening on HPLC could allow the implementation of secondary (morbidity) prevention to be planned in advance of the second semester of life, when the diseases will start manifesting. We also intended to study how primary retrospective and/or primary prospective prevention could be efficiently offered whenever an affected or a carrier neonate is detected and the parents are informed and referred to a genetic center for counseling and eventually prenatal diagnosis. Methods. We have created fresh artificial standard blood samples and we have used natural cord blood samples (CBS) to test the diagnostic confidence and the sample conditions before and after spotting aliquots on paper to be tested at increasing intervals of time up to a maximum of 3 weeks. Samples eluted from dry 3-mm paper discs were analyzed on HPLC, according to the manufacturer’s instructions and in several modified manners. Results were compared with the expected patterns for their diagnostic quality and stability. Results. All current abnormal Hb’s involved in SCD were identified using the artificial standard blood samples. In addition 94 natural CBS were analyzed on which we were able to identify Hb Bart’s, and HbS traits. DNA analysis confirmed the association of Hb Bart’s to a -α1 deletion. The samples spotted on paper degenerated rapidly. However, the interpretation of the results was still reliable on 15 days old dry samples, which period falls well within the boundaries of the screening program. The integration system of the Vnbs is not measuring the HbA1c with the precision necessary to make an educated prediction on a possible future hematological disorder. We are testing at this moment possible alternatives. Summary/Conclusions. The (Vnbs) HPLC apparatus recognizes with sufficient confidence all common Hb variants in heterozygous and homozygous state including HbS/S (SCD) and b-thalassemia major. This will enable pre-symptomatic genotype/phenotype determination and treatment planning for both diseases with a considerable gain in morbidity prevention and state of the art treatment. Moreover, obligatory or potential couple at risk can be immediately referred to a genetic centre for analysis, counseling and eventually primary prevention in a following pregnancy.

**1333**

**A SIMPLE, ACCURATE METHOD FOR THE ESTIMATION OF THE ERYTHROCYTE SEDIMENTATION RATE**

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Backgrounds. measurement of the erythrocyte sedimentation rate (ESR) is a helpful indicator of the presence and extent of inflammation and its response to treatment. ESR is influenced by proteins of acute phase response and anemia which may be present in such situations. Aims. and we have used natural cord blood samples (CBS) to test the diagnostic confidence and the sample conditions before and after spotting aliquots on paper to be tested at increasing intervals of time up to a maximum of 3 weeks. Measurements of their ESR were recorded every ten minutes. Results. by using the control measurement of the ESR at 20 minutes, the blood samples were classified into three homogeneous groups (group-1: ∆0-5 mmHg at 20 min, group-2: 6-10 mmHg at 20 min, group-3 more than 10 mmHg at 20 min) and a family of regression curves was fitted to the empirical data describing the relationship of the ESR on time. The linear model was chosen as the simplest with good fitting precision in the study. Conclusion: the constructed linear curves within the established groups of the patients enable the estimation of the ESR values at 60 minutes period, with only one measurement at 20 minutes.

Figure 1. ESR1.

**1334**

**NOVEL AND MEDITERRANEAN MUTATIONS IN β THALASSEMIA TRAIT INDIVIDUALS FROM NORTHERN IRELAND**

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1Belfast City Hospital, BELFAST, United Kingdom; 2Royal Victoria Hospital, BELFAST, United Kingdom; 3Ulster Hospital, DUNDONALD, BELFAST, United Kingdom; 4Queen’s University, BELFAST, United Kingdom

Summary/Conclusions. The (Vnbs) HPLC apparatus recognizes with sufficient confidence all common Hb variants in heterozygous and homozygous state including HbS/S (SCD) and b-thalassemia major. This will enable pre-symptomatic genotype/phenotype determination and treatment planning for both diseases with a considerable gain in morbidity prevention and state of the art treatment.
Backgrounds. Hemoglobinopathies, which include β thalassaemia, are a common group of genetic disorders prevalent in tropical and Mediterranean regions. They are co-incident with malaria suggesting that these disorders provide a selective advantage in malaria endemic areas. β thalassaeemia is characterised by deficient or absent synthesis of the β globin protein arising due to either point mutations, of which more than 200 have been described, or deletions of the β globin gene. Many of the common β thalassaemia mutations associated with severe thalassaemia major have a particular geographical distribution and haplotype analysis indicates they have common origins. Intriguingly, a low frequency of β thalassaemia mutations have been described in non-tropical populations such as Britain and although most of these mutations are of non-native origin, some are novel. Previously several Irish cases of β thalassaemia have been documented and a number of individuals with β thalassaemia trait have been identified in the County Down region of Northern Ireland but the molecular basis in all cases has not been investigated. Aims. To discover if the β thalassaemia trait in County Down was associated with common or unique mutations and to perform haplotype analysis to indicate the origin of the common mutations detected. Methods. DNA samples from 23 individuals were screened for base changes in the β globin gene using PCR-direct sequencing. Haplotype analysis was performed using seven polymorphic sites of the β-globin gene cluster on chromosome 11. Markers were amplified by PCR and products were analysed by restriction digestion. Haplotypes were reconstructed according to Orkin et al. (Nature 396 (1982) 627). Results: Sequencing the β-globin gene revealed that fourteen individuals possessed two common Mediterranean mutations, a C to T change at codon 59 in exon 2 and a G to A change at base 110 in intron 1. Both mutations were present on Haplotypes I, indicating a non-native origin. A further group of seven individuals shared a G to A change at base 850 in intron 2. This mutation has been previously described in an American family of Scottish-English descent (Curuk et al. Hematology 1995, 49.207). Finally, two novel β-thalassaemia mutations were detected. The A to C change at the initiation codon would cause defective mRNA translation, while the deletion of G from the last base of codon 109 would result in frameshift mutation. Summary. The two Mediterranean mutations, having arisen on Haplotype I, are of non-native origin and may have been introduced into the Northern Ireland population as a consequence of European trade. It is probable that the IVS2-850 (G to A) detected in this study shares a common origin with the family described by Curuk et al. Finally, it remains to be confirmed if the novel mutations have arisen de novo in Northern Ireland.


table

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1336

WARM AUTOIMMUNE HEMOLYTIC ANEMIA (WAHIA) FOLLOWING RECURRENT MYCOPLASMA PNEUMONIA INFECTION IN A CHILD WITH DOWN SYNDROME

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Backgrounds. Cold agglutinine syndrome (CAS) with cold IgM anti-I autoantibody represents a common hematological complication after Mycoplasma Pneumonia (MP) infection due to molecular mimicry. In only two cases both cold IgM and warm IgG autoantibodies have been identified. We present the first case of Autoimmune Haemolytic Anaemia (AIHA) following MP infection, with only warm IgG autoantibodies.

Case Report. An 8 years old boy with Down Syndrome (DS), presented with MP infection. The patient in the last 2 years presented with recurrent episodes of MP infection, followed by non immune hemolytic anemia with DAT(-) and normal cold agglutinine titer. The patient was treated with clathromycin. Four days after admission, laboratory findings of hemolysis was present: Hb 3g/dl, Ht 29.6%, LDH 1200U/ml, reticulocyte count 5%, haptoglobin 8mg/dl. Haemolysis was refractory to the initial treatment with α-globulin but was responded to predinisolone. The patient was followed up and presented additional MP infections, followed by WAHIA. The investigation of hemolysis was made by the ID-System of Dia-Med Company. The blood group of the patient was A Rhesus(+) cee, Kell(+), I antigen (+) positive. In the latest episode the immunohaematological results were as follows: Direct Antiglobulin Test (DAT): polyspecific(+),sops, anti-IgG (+),titer anti-IgG: 1/100(+), anti-C3d(-)neg, anti-IgA(-)neg, anti-IgM(-)neg, indirect Antiglobulin Test (IAT): (+) positive, owing to the presence of autoantibody. The serum and elution reacted with all red cells only at 37oC, in antiglobulin phase and the autoantibody was characterized as warm IgM panagglutinable while the cold agglutinin titer was <1/64. The findings from DAT and IAT at the follow up were the same but the titer of anti-IgG was higher 1/400. Finally the elution study revealed an IgG autoantibody with anti-e specificity. Autoimmunity studies were negative for ANA, AMA, ASMA, anti-DNA, whereas antithyroglobulin was positive. The serum immunglobulines and the protein electrophoresis were normal. The CD4/CD8 ratio was low (0.45). IgM antibodies for MP were positive. However, antibodies for CMV, EBV, Rubella, HSV, VSV and Chlamydia were negative. Summary/ Conclusions. After MP infection, even with antibiotic treatment, the patient may remain chronic carrier. High incidence of MP infection and severe manifestations is observed in DS because of immune abnormalities like defective cellular adhesion and ineffective lymphocyte activation, due to indegrin (LEA-1) dysfunction. Excluding concomitant infections, drugs and hereditary factors, the fact that WAHIA was not a unique random episode but was recurrently following MP infections, indicates that MP may trigger WAHIA like other known autoimmune phenomena e.g asthma, Polyneuropathy, urticaria and Guillain Barre Syndrome. In DS, there are complex thymic alterations, that could be correlated with the presence of autoactive CD4+T-cell, TH1/TH2 imbalance and early senescence of immune system resulting to early appearance of autoimmune phenomena like hypothyroidism, coeliac disease and IDDM. A polyclonal B-cell immune activation after a chronic MP infection in conjunction with a defective T-cell immunity that has been described in the genetic disorder of DS, may lead to WAHIA with warm IgG antithrocytic autoantibody formation, instead of CAS that is usually seen post to MP infection.
1337
STUDY OF ERYTHROCYTE MEMBRANE PROTEINS BY SDS-PAGE 10 YEARS EXPERIENCE
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Backgrounds. In the majority of Hereditary Spherocytosis (HS) and Hereditary Elliptocytosis (HE) cases the diagnosis can be made on the basis of clinical/ family history, red cell indices and osmotic fragility screening test. Quantification of erythrocytes membrane protein electrophoresis by SDS-PAGE can be useful in complex cases.

Objectives. To identify the most common protein defects in our population and to analyze the usefulness of erythrocytes membrane protein electrophoresis in the diagnosis of patients with anemia and/or hemolysis of unknown or multiple etiologies, we made the retrospective analysis of 584 non familial cases. Materials and Methods. 584 EDTA blood samples from Centro Hospitalar de Coimbra and from other Hospitals in Portugal and Spain. SDS-PAGE was performed according to J. Delaunay protocols. The reasons for the study were divided in 12 groups as listed on the Table. Results. 63% of HS cases have combined Ankyrin/Spectrin/Pr 4.2 deficiencies, 84% combined Band 3/Pr 4.2 deficiencies and in 4% a single Pr 4.2 reduction was detected. Pr 4.1 reduction was found in 56% of the HE cases and the remainder 44% had Spectrin α-β reduction. In 14 cases with HA of multiple etiology we detected Spectrin α-β reduction in four and Pr 4.1 reduction in seven. Among the 48 samples with HA of unknown etiology, one was HS and four were HE. In 18 samples referred as possible CDAs, two had Band 3 abnormal mobility. In the EMPD, SAO and AHAI the electrophoretic profile was similar to the normal controls. No abnormalities were observed in the group of samples referring investigation of anemia Conclusion: In HS and HE the relative percentage of protein deficits involved are similar to the described for other European populations (Delaunay et al., 1995, Eber et al., 1996). In our experience, SDS-PAGE electrophoresis can be useful for the diagnosis of CDA type II, when an EMPD is suspected, in HA of complex etiology and when the results of screening tests are equivocal or borderline or the clinical phenotype is heterogeneous among the affected family members. If no spherocytes or eliptocytes are found in peripheral blood smears, erythrocytes membrane protein electrophoresis carries no benefit.

1338
SPECTRUM OF ANTINUCLEAR ANTIBODIES IN SICKLE CELL DISEASE PATIENTS FROM OMAN
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Sultan Qaboos University, MUSCAT, Oman

Backgrounds. Sickle cell disease (SCD) is a significant public health problem in the Sultanate of Oman. Although rare, it is not infrequent to find the SCD associated with systemic lupus erythematosus (SLE) or other connective tissue disorders. Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease characterized by a variable clinical picture, a large range of clinical and serological manifestations and a relapsing-remitting course. The disease is very variable in severity. The complexity of the clinical picture of SCD can be increased by the simultaneous presence of manifestations attributable to disease activity, chronic damage, co-morbidity, especially infection or the co-existence with SLE.

Results. 3 abnormal mobility. In the EMPD, SAO and AHAI the electrophoretic profile was similar to the normal controls. No abnormalities were noticed in 10/107[9.35%] normal subjects and 6/35[17.2%] sickle cell trait subjects as controls. Results. A total of 67 patients [31 males;36 females] with the mean + SD age of 24.6±7.9yrs [Range 11-47] formed the study group. ANA was documented to be positive in 16/67 cases [24%] amongst the SCD patients. ANA positivity was noticed in 10/107[9.35%] normal subjects and 6/35[17.2%] sickle cell trait subjects. 6 of the 16[37.5%] SCD patients satisfied the revised classification criteria [minimum 4] for SLE of the American College of Rheumatology. Three patients had lupus anticoagulant, five had ACA positivity, one had Bechets, one had anti-thyroid antibodies and one had anti-red cell antibodies. Discussion. The study has demonstrates the prevalence of ANA positivity in normal subjects, sickle cell trait subjects and patients with SCD in Oman. The overall ANA positivity was observed to be about 24% which is considerably high. The prevalence was also twice as high in females as seen in the males. Furthermore, a significant number of these patients also had multiple autoantibodies.

1339
LYMPHOID EXTRAMEDULLARY BLAST CRISIS OF CHRONIC MYELOID LEUKAEMIA SIX YEARS AFTER ALLOGENIC TRANSPLANTATION
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Extramedullary disease (EMD), also called granulocytic sarcoma, following allogeneic hematopoietic stem cell transplant (allo-HSCT) in patients diagnosed with chronic myeloid leukaemia (CML) is an infrequent event. According to a retrospective analysis performed by the European Group for Blood and Marrow Transplantation the incidence rate for this complication was 0.22%. Considering that lymphoid transformation accounts for only 20-30% of all blast crisis events in CML, lymphoid EMD remains a relatively rare phenomenon. Reviewing the literature we found only four cases of lymphoid EMD relapse after an
The erythrogram was typical in 17/21 patients with RS and in 0/30 patients with No RS (p<0.0001). The positive predictive value for sideroblastic changes was 100% and the negative predictive value was 88%. Despite the RBC indices comparison showed statistical significance in some variables, they were no specific enough to identify sideroblastic changes. In the group with RS, mean cell hemoglobin was lower (median 30.8 versus 33.6 fl), RBC distribution width was higher (19.3% versus 16.5%), the percentage of hypochromic RBC was higher (5.3% versus 0.9%) and hemoglobin content of reticulocytes was lower (53 versus 37 pg) as compared to No RS patients (p<0.05). This last index was useful to rule out iron deficiency in RS group as a cause of hypochromic RBC changes, since in contrast to iron deficiency it was not decreased.

In conclusion: Sideroblastic changes can be recognized in the erythrogram. Indeed, despite myeloid neoplasia with RS are a heterogeneous group of diseases they have a common pattern of RBC distribution that can be considered as a kind of fingerprinting for sideroblastic changes with a high predictive value allowing a straightforward diagnostic approach in clinical practice.

1341 EPIDEMIOLOGICAL DATA ON MYELOID/PLASMYCOSIS SYNDROME PATIENTS FROM A ROMANIAN SINGLE CENTER
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Background. Since the World Health Organization (WHO) recognized MDS as a disease entity only starting with 1997, epidemiological data in MDS cannot be retrieved from official statistics on morbidity and mortality and have to be extracted from specialized registers. We present the first Romanian study on the incidence and characteristics of MDS, based on the data existing in Fundeni Clinical Institute, Bucharest, the greatest hematological department in Romania. Method. The MDS files at diagnosis of the patients admitted during the period 1980-2005, recorded in the registration forms provided by the MDS Foundation (USA), represented the primary data-base. The hematological data of the MDS patients included in the registry were re-evaluated and classified according to French-American-British (FAB) criteria. The distribution by sex, age groups, subtypes and the annual number of new cases were analysed comparatively with other reference studies. Results. Four-hundred and twenty four cases of MDS were identified. The distribution between sexes was relatively balanced with a slight global preponderance of males (M/F 1.26), except for refractory anemia with excess of blasts (RAEB) 1.94. The mean age at diagnosis was 62.5 years (16-90). Most of the patients (60.6%) belonged to the group of age 61-80, where all the subtypes of MDS had the highest rates. A noticeable proportion (17%) had ages below 50 years, 25% of which in the range 16-30. On the other hand, few cases (4%) were above 81. Patients with refractory anemia (RA) and refractory anemia with ringed sideroblasts (RARS) accounted for 44.5% of all cases (RA 29%, RARS 15.5%), RAEB and RAEB in transformation 35%, chronic myelomonocytic leukemia 5.6% and unclassified 16.7%. The annual number of new cases was constantly low during the period 1980-1989, but increased dramatically from 11 cases/year in 1990 to a maximum of 48 cases/year in 1999, showing a
certain decrease afterwards. The subtypes with the most important increase in time were RA and RARS. Conclusions: This study indicates an actual increase of the number of MDS cases in Romania over the investigated period of time. Particularly, a noticeable proportion of young patients and a low proportion of patients ≥ 81 years have been found, which make our findings closer to the Asian than to the Western MDS epidemiological results.

**1342**

**SINGLE PEGFILGASTRIM INJECTION AFTER FLUDARABINE-CYTARABINE BASED REGIMENS FOR TREATMENT OF POOR PROGNOSIS MYELODISPLASTIC SYNDROME (MDS) AND ACUTE MYELOID LEUKEMIA (AML): PRELIMINARY DATA ON HAEMATOLOGIC RECOVERY**

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San Raffaele Scientific Institute, MILAN, Italy

Backgrounds. Response of poor prognosis MDS and AML to conventional chemotherapy (CT) is unsatisfactory. Regimens comprising Fludarabine and Cytarabine (FLA), with or without Idarubicin, have shown promising results in induction of complete remission (CR), with a favourable toxicity profile. In FLA regimens filgrastim is administered from day 0 to day 5 to induce cell cycling and therefore sensitization to CT, then from day 12 to enhance recovery of neutrophils. Peg-filgrastim is a covalently bound conjugate of filgrastim and monomethoxypolyethylene glycol. It has a longer elimination half-life than the unconjugated filgrastim because of decreased serum clearance. After standard chemotherapy for non-myeloid malignancies, one dose of Peg-filgrastim showed to be equivalent to daily filgrastim in enhancing neutrophil recovery, and the single injection was largely preferred by patients (pts). From March 2005, in MDS/AML pts we started to administer a single dose of Peg-filgrastim at day 12 of FLA regimens instead of daily unconjugated filgrastim. Aim: to evaluate the efficacy and cost-effectiveness of a single Peg-filgrastim injection given at day 12 from the beginning of FLA regimens, in poor prognosis MDS and AML pts. Methods. From March 2005 to December 2005 13 FLA cycles with Peg-filgrastim s.c. injection at day 12 have been administered to 10 pts, at our Institute (Group PEG); neutrophil and platelet absolute count have been monitored daily from day 0. Data on haematologic recovery after 53 FLA cycles with unconjugated filgrastim (dosage: 300 mcg/sm2/day) in 36 pts, period January 1999-February 2005, have been retrieved from our database (Group NO-PEG). Filgrastim has been administered until neutrophil count > 500/mm3. Group PEG: median age 66 (range 49-73); diagnosis of MDS=3, AML=6, granulocytic sarcoma=1; status pre-FLA: CR1=5, CR2=1-2, NOCR=6. Group NO-PEG: median age 56 (range 22-69); diagnosis of MDS=22, AML=14, granulocytic sarcoma=0; status pre-FLA: CR1=16, CR2=1-0, NOCR=37. Results. Data on haematologic recovery are shown in the table.

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<td>Platelets &lt;20000/mm³ (n° of days)</td>
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<td>Platelets &gt;20000/mm³ (day from start of CT)</td>
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n.e.=not evaluable.

A single injection of Peg-filgrastim has been administered in 12 out of 13 cycles in Group PEG; in one case a second injection has been administered at day 32, for delayed recovery. The mean number of vials per cycle of unconjugated filgrastim administered to Group NO-PEG has been 4.7. Apoptosis in the low risk group was higher but not significant compared to that of high risk group (p=4.3 in the RAEB, RAEB-t and CMML). Caspase 8 was expressed in 14/31, caspase 3 in 15/31, caspase 6 in 15/31, caspase 7 in 3/31, caspase 9 in 2/31, caspase 10 in 1/31 and caspase 2 in 1/31 cases in 14/31 cases including all FAB subtypes with the exception of caspase 3 that was not expressed in any of the 7 RA cases examined. The level of caspases and granzyne B expression did not correlate with different hematological parameters or the values of apoptosis. Moreover, cases with ratio of granzyne B gene expression compared to that obtained from the normal pool RNA. A ratio of positive cases for all genes examined was not significantly different between different FAB subgroups and different IPSS risk categories. The level of caspases and granzyne B expression did not correlate with different hematological parameters or the values of apoptosis. The level of expression as well as the percentage of positive cases for all genes examined was not significantly different between different FAB subgroups and different IPSS risk categories. The level of caspases and granzyne B expression did not correlate with different hematological parameters or the values of apoptosis. Conclusions: Dependent on the IPSS risk category, the FAB subtype and the level of apoptosis. Caspase1,2,3,5,6,8,9 were expressed in the bone marrow of adult de novo MDS in 38-58% of cases including all FAB subtypes. Caspase 5 was less frequently expressed and was negative in all RA cases examined. The level of caspases and granzyne B expression did not correlate with different hematological parameters, FAB classification, the IPSS risk group and the level of apoptosis. Larger number of cases need to be examined to draw definite conclusion about the role of these apoptosis regulatory genes in the pathogenesis and prognosis of MDS.

**1343**

**ANALYSIS OF CASPASES GENES EXPRESSION IN THE BONE MARROW OF ADULT DE NOVO MYELODYSPLASTIC SYNDROMES (MDS)**

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Backgrounds. Myelodysplastic syndromes cover a range of clonal stem cell disorders characterized by ineffective hematopoiesis which has been associated with excessive intramedullary apoptosis of hematopoietic cells. Caspases constitutes a family of cystosolic proteases which are the effecter molecules of apoptosis. Aim: The aim of the present study was to examine caspases and granzyme B expression and the degree of apoptosis in the bone marrow of adult de novo myelodysplastic syndromes and to correlate our findings with clinical parameters and prognosis.

Methods. We studied 31 cases of MDS including 7 RAEB-t, 9 RAEB, 4 CMML, 7 RA and 4 RARS according to FAB criteria. The degree of apoptosis was determined by flow cytometry using the Annexin method on fresh bone marrow mononuclear cells. mRNA was extracted and the expression of caspases 1, 2, 5, 6, 7, 8 and 9 and granzyme-B was determined using a multiprobe RNase Protection Assay System (Riboquant, BD Biosciences). A pool of RNA from normal bone marrow mononuclear cells was used as a normal control. The expression of each gene was compared to that of two housekeeping genes (GAPDH and L32) using the Image Master analysis Software. The level of each gene expression was compared to that obtained from the normal pool RNA. A ratio was obtained and two groups were generated with values > or <1. The expression of the genes and the degree of apoptosis were analyzed taking into consideration haematological parameters, the FAB classification and the IPSS value. Results. The median value of apoptosis for all MDS cases was 4.7. Apoptosis in the low risk group was higher but not significantly different than that of the high risk group (p=4.3 in the RAEB, RAEB-t and CMML). Caspase 8 was expressed in 14/31, caspase 3 in 15/31, caspase 6 in 15/31, caspase 7 in 3/31, caspase 9 in 2/31, caspase 10 in 1/31 and caspase 2 in 1/31 cases including all FAB subtypes with the exception of caspase 3 that was not expressed in any of the 7 RA cases examined. The level of caspases and granzyne B expression did not correlate with different hematological parameters or the values of apoptosis. Moreover, cases with ratio of granzyne B gene expression compared to that obtained from the normal pool RNA. A ratio of positive cases for all genes examined was not significantly different between different FAB subgroups and different IPSS risk categories. The level of caspases and granzyne B expression did not correlate with different hematological parameters or the values of apoptosis. Conclusions: Dependent on the IPSS risk category, the FAB subtype and the level of apoptosis. Caspase1,2,3,5,6,8,9 were expressed in the bone marrow of adult de novo MDS in 38-58% of cases including all FAB subtypes. Caspase 5 was less frequently expressed and was negative in all RA cases examined. The level of caspases and granzyne B expression did not correlate with different hematological parameters, FAB classification, the IPSS risk group and the level of apoptosis. Larger number of cases need to be examined to draw definite conclusion about the role of these apoptosis regulatory genes in the pathogenesis and prognosis of MDS.
BM lymphocytes of patients with MDS, as there is a controversy between research groups regarding the lymphoid lineage participation in the pathogenesis, diagnosis and/or in the prognosis of MDS. Following the new classification of MDS by the World Health Organization (WHO) it seems challenging and interesting to investigate, apart from MDS, a group of patients with CMML, which has been classified by WHO as the new group of Myelodysplastic/Myeloproliferative Disorders (MDS/MPD). Methods: BM samples from 32 patients with MDS (n=15), MDS/MPD (n=12), and MDS/AL (n=7) and 5 BM from healthy individuals, as a control group, were analyzed by multiparametric 3-color flow cytometry using an extensive combination panel of monoclonal antibodies (CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11b, CD11c, CD13, CD14, CD16, CD19, CD20, CD33, CD34, CD46, CD56, CD64, CD66, CD68, CD117, HLA-DR, KORSA, MPO/LF and TdT) in the gate of the lymphocytes by the histogram of SSC=1 (expression of CD45). Results. The MDS patients were characterized by the following statistical significant findings in comparison to the control group: a) a decrease of the lymphocytes mean SSC (59,42±5,62 vs 87,60±22,52, p=0,001) and b) a decrease of the fluorescence intensity of CD45 in lymphocytes (43,98±15,99 vs 65,64±17,12, p=0,035). The MDS/MPD patients compared to the control group were characterised by: a) a decrease of the CD38+ (31,01±25,87 vs 42,34±25,06, p=0,013) and b) a decrease of CD56+ (5,80±6,69 vs 20,45±16,72, p=0,056) lymphocytes percentage. The MDS group in comparison to the MDS/MPD group showed a statistical significant decrease of the lymphocytes mean SSC (59,42±5,62 vs 70,83±17,02, p=0,014) along with a decrease of the percentage of co-expression of CD3/CD16/CD56 (5,80±3,86 vs 11,58±6,72, p=0,026). When MDS/MPD group was compared with MDS/AL a decrease of 1 lymphocytes (CD2+ 58,59±3,80 vs 88,06±5,57, p=0,009) and an increase of B lymphocytes (CD19+ 12,73±5,23 vs 7,34±6,08, p=0,004 and CD19+ 12,67±3,55 vs 7,36±5,64, p=0,009) were observed. It should be noted that myeloid markers expression in the lymphoid populations didn’t show any differences. Summary/Conclusions. The above-mentioned statistical significant findings indicate the importance of further study of this cell lineage in MDS and MDS/MPD cases to answer several questions such as is the decrease of lymphocytes side scatter and CD45 expression indicative of lymphocyte immaturity? As this is an ongoing study, more cases will possibly clarify the disturbances of lymphocytes and their significance in this group of patients.

We have studied the methylation status of the differentially methylated region (DMR) of GTL2 promoter in order to detect epigenetic alterations of DLK1/GTL2 gene. Methods. We have studied 8 patients, 6 males and 2 females, with myelodysplastic syndromes (MDS) classified according to the FAB system; 2 patient with RA (25%), 3 with RAEB (37.5%), 2 with RAEB-T (25%), and 1 with CMML (12.5%). Median age was 68-4 years (range 38-88). Cytogenetic analysis: 2 patients had chromosomal abnormalities, and 4 patients had apparently normal karyotypes, 1 had 45,XX,-7 karyotype , and 1 had 47, XX,+8 karyotype . None of the patients had ever received therapy with hypomethylating agents. DNA methylation pattern was determined by methylation-specific PCR of samples previously subjected to bisulphite-treatment, according to preestablished procedures. Subjects who have undergone bone marrow aspiration for diagnosis of thrombocytopenia, and after we had excluded hematological malignancies, served as controls. Results. We have studied the methylation pattern in both blood and bone marrow. The normal pattern consists of 2 bands (alleles), namely one corresponding to the methylated paternal allele, (size 160 bp) and one corresponding to the unmethylated maternal allele (size 120bp). We have found that alterations of the DMR were present in 4 (50%) of the patients studied: 2 (25%) had an abnormal methylation pattern in both blood and bone marrow samples and 2 (25%) others presented the same abnormal methylation pattern only in blood samples. No alteration of the methylation pattern was observed in the remaining 6 bone marrow samples. In the remaining 4 samples only the methylated allele was present. Summary/Conclusions. It is known that DLK1 gene is overexpressed in patients with MDS. A total of 16 samples were studied and 6 (57.5%) were found to be abnormal. It is probable that LOI through epigenetic modifications in the DMR of the GTL2 gene represents a potential pathogenetic mechanism in MDS. The objective of the present study was to examine of the DMR of the DLK1/GTL2 gene in MDS patients and compare it with typical MDS and MPD. The results have been statistically tested by using T-student test for the comparison of data. The results have been tested in order to verify our preliminary findings and to study further the imprinting status of the gene.

**Figure 1. Lymphocytes gating of MDS/MPD BM sample.**

**1346**

**COMPARISON OF IN VITRO GROWTH OF GRANULOCYTE-MACROPHAGE COLONY (CFU-GM) FORMATION IN PATIENTS WITH MYELODYSPLASTIC/MYELOPROLIFERATIVE DISEASES (MDS/MPD), TYPICAL MYELODYSPLASTIC SYNDROMES (MDS) AND MYELOPROLIFERATIVE DISORDERS (MPD)**

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Myelodysplastic/myeloproliferative disorders (MDS/MPD) are a new category of disorders, which is separated by WHO classification. This group consist of 4 type of disorders: Chronic Myelomonocytic Leukaemia (CMML), atypical Chronic Myelogenous Leukaemia (aCML), Juvenile Myelomonocytic Leukaemia (JML) and Chronic Myelodysplastic/Myeloproliferative disease-unclassifiable (MDS/MPD-U). We still lack understanding of disturbances of hematopoiesis in patients (pts) with MDS/MPD. The objective of the present study was to examine of the hematopoiesis in pts with MDS/MPD compared with typical MDS and MPD in vitro. We enrolled 36 pts (25 pts with RA-4, RARS-2, RCMD-17, RCMD/RS-7, 5q(-)-5, RAEB1-4, RAEB2-7, MDS-U)-2, 12 pts with MPD (CML-5, OMF-4, CMPD-2) and 25 pts with MDS/MPD (aCML-6, CMML1-3, CMML2-6, MDS/MPD-U). Human CFU-GM cells were cultured by plating 1 x 10⁵ mononuclear cells to semisolid methylcellulose medium without or with cytokines (GM-CSF or G-CSF or SCF+GM-CSF+IL-3+Epo). CFU-GM colonies were scored at day 14. We compared spontaneous growth of CFU-GM and in presence of cytokines between patients with MDS/MPD, MDS and MPD. All of the results have been statistically tested by using T-student test for the independent groups. For statistically significant results were p<0,05. In pts with MDS/MPD compared to pts with typical MDS the spontaneous growth (respectively: med. 2 vs 0; p=0,0042), the growth with G-CSF (respectively: med. 13 vs 3; p=0,0016) and the growth with GM-CSF (respectively: med. 83 vs 16,5; p=0,042) of CFU-GM were statistically significant higher. In pts with MDS/MPD according to pts with MPD: the spontaneous growth (respectively: med. 2 vs 76; p=0,0065) and the growth with G-CSF (respectively: med. 13 vs 146; p=0,0042), with GM-CSF (respectively: med. 83 vs 319; p=0,010) and with GM-CSF+IL-3+Epo (respectively: med. 68 vs 224; p=0,010) of CFU-GM were statistically significant lower. The growth of CFU-GM in pts with CMML1 was statistically significant lower according to pts with CMML2 in culture with G-CSF (respectively: med. 5 vs 184,5; p=0,031) and with GM-CSF (respectively: med. 17,5 vs 341,5; p=0,023). Statistically significant differences in culture of CFU-GM between pts with: MDS/MPD, typical MDS and MPD verify distinct biology of MDS/MPD. Statistically sig-
significant differences in growth of CFU-GM in culture with G-CSF and with GM-CSF between pts with CMML and CMMML show another biology isolated by WHO classification subtypes of CMML.

1347
BLAST CELL COUNT IN THE BONE MARROW OF PATIENTS WITH MYELODYSPLASTIC SYNDROME (MDS) OR SECONDARY ACUTE MYELOID LEUKAEMIA (sAML): COMPARISON BETWEEN MORPHOLOGIC ASSESSMENT ON MARROW ASPIRATE (AS) AND IMMUNOHISTOCHEMISTRY ON BONE MARROW BIOPSY (BMB)

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Backgrounds. in the French-American-British (FAB) co-operative group Congress of the European Hematology Association According to low correlation obtained between CD117 and CD34+ blasts by immunohistochemistry on BMB in MDS and sAML compared to AS.

Results. We reviewed the marrow aspirates and core biopsies of 169 pts with MDS or sAML at diagnosis, period 1997-2005. Marrow blasts have been morphologically quantified on May-Grunwald Giemsa stained AS and expressed as blast percentage over 500 nucleated marrow cells. Bionin’s fixed, paraffin-embedded BMB have been evaluated for CD34+ immature cells counted over 1000 nucleated marrow cells. Diagnoses (FAB) according to morphology of marrow aspirate were: RA=116, RAE=34, RAEB=12, CMML=5, sAML=12. According to FAB, WHO and IPSS, marrow blasts percentages have been grouped into four classes: A=0-4, B=5-9, C=10-19, D=20. Each marrow AS and BMB from single patient has been assigned a class and compared. Results. 50 cases (29.6%) showed a difference in blast percentages, thus determining a class discordance; details are reported in the table. Most importantly a difference >1 class was observed in 10 cases (5.9%); in 5 cases blast cell count was higher and in the remaining 5 lower on BMB when compared to AS. Conclusions. This large retrospective mono-institutional study highlights the necessity to perform blast cell count both in AS and BMB of patients with suspected MDS/sAML at diagnosis. Immunohistochemistry for CD34 on BMB is useful in those cases with low (< 10%) blast count, allowing a reliable blast cell count also on BMB.

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1348
BCR-ABL/ABL RATIO AND FISH PH POSITIVITY - RELATION TO C-KIT EXPRESSION AND DUAL ESTERASE ACTIVITY IN CML PATIENTS ON GLEEVEC THERAPY

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Backgrounds. Chronic myelogenous leukemia (CML) is a myeloproliferative disease with t(9;22) and/or BCR/ABL fusion gene. One of treatment options for CML patients is inhibition of BCR/ABL and c-kit (CD117) tyrosine kinase activity with imatinib mesylate (Gleevec). Aim of the study: To analyze CD117 expression and dual esterase activity in bone marrow hematopoietic cells (HCS) of CML patients on Gleevec therapy and to correlate the results with percentage of FISH Ph positive HCs and bcr-abl/abl ratio.

Methods. Bone marrow aspirates and core biopsies of 17 CML patients on Gleevec therapy (duration of therapy 4 - 32 months) were analyzed by FISH and quantitative RT-PCR, immunocytochemical APAAP CD117 expression and cytochemical dual esterase activity. Patients were divided in subgroups according to duration of therapy (less than 6, 6-12 and more than 12 months). Results. Patients with leukocyte count > 40 x 10^9/L (70%) had high bcr-abl/abl ratio, FISH Ph positive HCs and CD117 positive HCs. Medians of CD117 and FISH Ph positive HCs were highest in CML patients during the first 6 months of Gleevec treatment, but correlation of these two parameters was low (0.19). There was no statistical difference when medians of CD117 and dual esterase positive HCs were compared between subgroups. Correlation of FISH Ph positive HCs and bcr-abl/abl ratio was high (0.68) with constant decrease in percentage of FISH Ph positive HCs and bcr-abl/abl ratio during follow-up. Conclusions. According to low correlation obtained between CD117 expression and dual esterase activity with percentage of FISH Ph positive HCs and bcr-abl/abl ratio, FISH and quantitative RT-PCR are the methods of choice for monitoring the efficiency of Gleevec therapy.

Hyperthermia causes a variety of morphological and functional effects in various cancer cells. At high temperatures, hyperthermia can induce differentiation in different tumor cells including leukemia cells, but severe treatments cause cell death by apoptosis or necrosis. Hyperthermia also affects heat shock protein gene expression. Since heat shock protein 70 (HSP70) has a crucial role in cell differentiation and cytoprotection, this protein may have a new role in differentiation and apoptosis induced by hyperthermia in K562 erythroleukemia cells. In the present work we have studied the effects of mild and severe treatments on differentiation induction and apoptosis in K562 cells. For this purpose, differentiation and apoptosis were measured along with the level of HSP70 protein. Erythroid differentiation was measured by benzidine staining assay and analyzing the expression of glycophorin A by flow cytometry technique. Apoptosis was evaluated by flow cytometric method based on binding of Annexin V and DNA staining by PI. DNA fragmentation was also studied. HSP70 protein level was determined by HSP70 ELIZA kit. Our results showed that mild hyperthermia (44oC) reduced cell growth and induced differentiation without affecting cell viability but heating cells at 45°C reduced the viability and totally inhibited the growth of these cells and no sign of differentiation was observed. On the other hand, mild hyperthermia (43°C) had not significant effect on induction of apoptosis in these cells, while, 45oC temperature caused cell death by apoptosis and necrosis. The level of HSP70 protein increased in cells treated with 45°C compared to the control cells, while, no significant increase could be detected at 45°C. In conclusion, increase in HSP70 protein level in 45°C heated cells can cause cytoprotection and lead to their differentiation, while, severe treatment, which cause no increase in HSP70 protein level, may lead to apoptosis of these cells.

1350
MUCOSITIS IN PATIENTS WITH CHRONIC MYELOID LEUKAEMIA (CML) ON IMATINIB, AN INDIAN EXPERIENCE

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To evaluate the incidence and severity of mucositis in patients with chronic myeloid leukemia (CML) on Imatinib. Retrospective data analysis conducted at Kidwai Memorial Institute of Oncology, Bangalore, India, a tertiary care cancer center with an annual attendance of 16000 new cases. All patients of CML who were on Imatinib were analysed. They were stratified into chronic phase (CP), accelerated phase (AP), and blast crisis (BC). The CTC criteria was used to assess mucositis. A total of 210 patients with complete clinical data were analysed. Details are shown in Table 1.
The majority of patients (90%) in BC developed mucositis, while AP (60%), and CF (26%) had a lower incidence. Mucositis onset was within the first 3 months of initiating imatinib in the majority (87%) of patients (p value <0.01), however, no patient required dose reduction or cessation of therapy due to mucositis. The median time for resolution of mucositis was 6 weeks irrespective of the stage of CML.

**1351**

**IDENTIFICATION OF A RARE E6A2 BCR-ABL FUSION TRANSCRIPT IN CHRONIC MYELOID LEUKEMIA: A CASE REPORT**


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The balanced translocation (t(9;22)(q34;q11) producing the chimeric BCR-ABL transcript is the hallmark of chronic myeloid leukemia (CML). Commonly, the breakpoints in the BCR gene occur within the major breakpoint cluster region (M-bcr), producing different types of BCR-ABL transcripts (e13a2 and/or e14a2). Breakpoints outside M-bcr occur rarely, in either minor-bcr (m-bcr) or micro-bcr (µ-bcr) leading to e1a2 and e19a2 fusion transcripts, respectively. Atypical BCR breakpoints outside these cluster regions are very rare. In particular, five cases of Philadelphia (Ph) positive CML and one case of CMML in progression have been associated with an atypical e6a2 BCR-ABL fusion transcript. The breakpoint in bcr intron 6 has been implicated as the cause of a more aggressive clinical phenotype and of increased oncogenic potential, due to the partial loss of important regulatory BCR sequences. Clinical outcome of CML expressing e6a2 fusion transcript and imatinib efficacy are not well established. AIM: We describe clinical and molecular features of a new case with Ph+ CML expressing e6a2 fusion transcript. CASE REPORT: A 54-year old man was admitted to Surgery Unit because of aortic occlusive arteriopathy of the legs. His peripheral blood count showed mild anaemia (hemoglobin 10.7g/dL), thrombocytopenia (platelets 100x10^3/L, with 30% neutrophils, 41% lymphocytes, 7% monocytes, 17% eosinophils and 5% basophils). Ten days later the patient was referred to our Haematology Department because of persistent leukocytosis and eosinophilia; however, 4 months later, cytogenetic analysis showed only occasional weak staining in mononuclear cells undergoing treatment with imatinib and in normal donors. Gene expression was normalised against ABL as a control gene. Gene expression is reported as a percentage relative to ABL expression per 5 μL cDNA reaction. Western blotting and confocal microscopy was used to examine CCN3 protein levels in normal bone marrow samples and bone marrow from CML patients at diagnosis and following imatinib treatment. BCR-ABL burden in CML bone marrow (BM) samples at diagnosis was high (median 285.8%, n=11). In contrast CCN3 expression in these samples was low (median 0.9%). Patients with a complete cytogenetic response (CCR) and approaching molecular remission (less than 10 BCR-ABL transcripts (median 0.6%)) in follow-up peripheral blood (PB) showed a significant increase in CCN3 expression approaching that observed for normal PB. The median CCN3 increase was 16.3% for CML patients responding to imatinib (p=0.005) and the median follow-up period was 28 months (range 6-60); median CCN3 expression for normal PB is 0.15%. Western blotting showed high CCN3 protein expression in normal BM (n=3). CCN3 expression was weak or absent in 3 CML BM samples at diagnosis and returned to levels comparable with normal BM upon response to treatment. Similarly, CD34+ cells extracted from CML BM showed increasing CCN3 expression with imatinib treatment (1351) whereas in vitro median increase 47%, n=3). Confocal microscopy showed only occasional weak staining in mononuclear cells from CML patients at diagnosis. Upon entering complete cytogenetic remission, the majority of cells stained positively for CCN3 expression. CCN3 expression has a reciprocal relationship with BCR-ABL in CML cell lines and primary human CML cells. Imatinib treatment of CML cells increases CCN3 expression. CCN3 expression may prove to be a useful marker in monitoring patient response to imatinib.
induced 7-65% growth inhibition of NB4 cells. Cell viability was also decreased by 2-55% between 24 to 96 h treatments with the drug. These effects of the drug were also dose-dependent. 3-HK induced a significant G1-arrest up to 48 h which consequently followed with appearance of sub-G1 peak (apoptosis) at 72 and 96 h. In addition we confirmed that the inhibition of proliferation is associated with differentiation especially toward macrophage-like morphology. Conclusion. We showed that 3-HK is a potent differentiating and apoptotic agents. These results can introduce 3-HK as potent candidate to treatment of leukemia.

1354
THE ROLE OF MDR RELATED PROTEINS IN THE PROGNOSIS OF ADULT ACUTE MYELOID LEUKAEMIA (AML) WITH NORMAL KARYOTYPE
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Background. Cytogenetic abnormalities are among the most important factors affecting the outcome of patients with acute myeloid leukemia (AML), but approximately 40-50% of AML cases display a normal karyotype, and to design risk-adapted therapeutic strategies.

Aims. We have compared the expression of P-glycoprotein (PGE), multidrug-resistance related protein (MRP) and lung resistance protein (LRP) with the clinical and biological characteristics of 135 adult patients with normal karyotype AML, to evaluate their possible impact on response to therapy and on survival. Methods. Median age was 55 years and 60 out of 135 (44%) patients were older than 55 years. Therapy consisted of a standard 3/7 (idarubicin and cytarabine) course as induction and intermediate-dose cytarabine (2 courses) as consolidation for 38 patients. Fluorarabine-based induction course (ELA/I / FLAIE) followed by intermediate-dose cytarabine as consolidation was used in 65 patients. Thirty-two patients were included in a clinical trial comparing FLAI to ICE as induction course, followed by a consolidation course of high-dose cytarabine. For statistical analysis response to therapy was evaluated after two chemotherapy courses. Patients who underwent allogeneic stem cell transplantation were censored at time of transplant.

Results. Increased PGP expression was associated only to advanced age (p=0.003). Conversely, no difference in the two age cohorts was found in MRP and LRP expression. No association was assessed between PGP, MRP and LRP over-expression and clinical and biological characteristics. Complete remission was strongly affected by PGP over-expression. In fact only 13/34 (15%) PGP-negative, but 19/48 (44%) PGP-positive patients did not respond to chemotherapy (p = 0.006). Advanced age and CD34 positivity on blast cells confirmed their negative role on obtainment of remission. No impact on response to therapy was demonstrated for MRP and LRP. However, a lower percentage of complete responses was observed in those patients over-expressing more than one MDR protein (p=0.03). Event-free survival of the whole population was 9 months. In the univariate analysis DFS was influenced by PGP over-expression (10 vs 4 months, p=0.035). DFS was negatively affected also by age (p=0.0006) and CD34 (p=0.044). In multivariate analysis all this factors retained their statistical significance. Summary/Conclusions. In our study only PGP expression showed a negative correlation with response to induction therapy, as well on DFS. MRP or LRP did not influence treatment outcome when singularly considered, but patients over-expressing more than one MDR-related protein had a lower probability to achieve CR. Age was the most important factor affecting DFS, but shorter DFS duration was observed also in PGP-positive patients and in those with CD34 aberrant expression. Our data confirmed the prognostic role of MDR proteins also in the subset of AML patients with normal karyotype, and could be used to stratify patients with different prognosis and to design risk-adapted therapeutic strategies.

1355
THE EVALUATION OF ANTI TUMORAL AND DIFFERENTIATION OF PLANT-DERIVED AGENTS IN COMBINATION WITH ATRA ON LEUKEMIC CELLS
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Backgrounds. Acute leukemia is characterised by accumulation of neoplastic cells which fail to develop into mature cells. Cytotoxic, differentiation and apoptotic agents have been employed for treatment of leukemia. In iranian traditional medicine plant-derived agents have been used for treatment of cancer. Aims. The present study is evaluation of cytotoxicity, apoptosis and differentiation of several plant extracts such as Peganum Harmala, Harmine, Harmaline, Urtica Dioica, Chelidonium Majus and Viscum Album on HL60 cells. ATRA has been used as standard agent. However, little study have been reported using these agents on this cell line, these components were initiated such as an investigation. Methods. HL60 cells were cultured to cells and incubated for 5 days. Counting of cells, viability, MITT, morphology, NBT reduction and cytofluorometric analysis performed by FACS using PI for cell cycle ,markers including CD11b and CD14 for myeloid differentiation and apoptosis using Annexin V. Results. The data showed that all agents in optimal dose caused cessation of proliferation in dose and time dependent manner(p<0.05). Optimal concentration of Peganum Harmala, Harmine, Harmaline, Urtica Dioica, Chelidonium Majus and Viscum Album (10 μg/ml,1.6 μg/ml,10 μg/ml, 2.5 mg/ml, 0.1 mg/ml and 50 μg/ml respectively) were chosen as antiproliferative effect with good viability. However, all agents in higher concentration were cytotoxic. Treated cell with ATRA showed depletion of growth in optimal dose of 10-7 Ml. Cells accumulated in GI phase using ATRA (81.5%), Urtica Dioica (75%) and Viscum Album (72%) but they arrested in S phase using Peganum Harmala, Harmine, Harmaline (52.7%) and Chelidonium Majus (54.5%). Only, cells induced by Harmaline 10 mcg/ml showed myeloid differentiation with some morphological changes , NBT positivity (28%) and increase in CD11b (24.3%) and CD14 (42.5%) (p<0.05) compared to ATRA (40% as NBT, 71% and 5.7% as CD11b and CD14).However, Viscum Album showed some apoptotic changes in 100 mcg/ml concentration. The combination of these agents in optimal dose with ATRA did not show any effect on differentiation of cells and ATRA preserved effect of differentiation of itself with higher cessation of proliferation. Conclusions. In conclusion, these data showed that the combination of these plant extracts with cytotoxic and differentiation agents may open a new window in leukemic in vitro therapy which requires further investigation.
for heterozygous D104N genotypes, respectively). We have observed similar frequencies of D104N genotypes in AML patients and controls (14.8% and 13.7%, respectively; \(p=0.76\)). Similar risks for the disease were also seen in individuals with heterozygous D104N polymorphism in comparison with the wild genotype (OR=1.09, 95%CI: 0.61-1.96). Considering only the AML patients, no differences in the frequencies of D104N polymorphism were found according to gender (12.2% in male vs. 18.8% in female; \(p=0.44\)), age (10.8% in 75 patients under 50 years vs. 22.5% in 49 patients at an older age; \(p=0.07\)), and ethnic origin (15.1% in Caucasian patients vs. 12.5% in Black patients; \(p=1.00\)).

Conclusion: Our results present preliminary evidence that the D104N polymorphism of the COL18A1 gene may be an unimportant determinant of the AML susceptibility.

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OUTCOME OF ALLOGENIC STEM CELL TRANSPLANTATION IN ADULT PATIENTS WITH PH+ AML

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Backgrounds. The Philadelphia chromosome-positive (Ph+) acute myeloid leukemia (AML) is rarely found in adult patients with an overall incidence of less than 1%. Most patients with Ph+ AML have an extremely poor prognosis when treated by chemotherapy alone. Recently, allogeneic hematopoietic stem cell transplantation (HSCT) performed early during remission with an improved treatment agent, using an interim therapy of imatinib (Glivec, ST571); has suggested a long-term survival. We understand a role of Ph+ AML and envision the future treatment strategy, we analyzed the effect of imatinib addition into standard chemotherapy as an alternative before allogeneic HSCT for newly diagnosed Ph+ AML. Methods. Between Nov 2001 and Oct 2004, 12 (2.2%) of the 556 adults (age, 17~84) with AML were Ph+ at the time of diagnosis. Of these, 5 patients with newly diagnosed Ph+ AML who completed induction chemotherapy and received matched or mismatched allogeneic HSCT were investigated in this study. Overall complete remission rate was 58.3% (7/12) by intention-to-treat analysis. Two patients were excluded because of their refusal of further treatment. All the patients were treated according to our center's standard protocol, which consists of 8x10 idarubicin (IDA) plus N4-phenoxyl-1-b-D-arabinofuranosyl cytosine (BH-AC) induction chemotherapy. Patients who achieved CR after induction chemotherapy were routinely assigned to receive 400 mg or 600 mg imatinib daily. Subsequently, patients in CR received consolidation chemotherapy consisting of 3x5 IDA plus BH-AC followed by a second imatinib cycle bridging the time to HSCT. The preparative regimen consisted of total body irradiation (1320 cGy) and Busulfex (3.2 mg/kg for 2 days) for patients in first CR. Graft-versus-host disease (GvHD) prophylaxis was attempted by administering cyclosporine or tacrolimus plus methotrexate. Stem cell source was bone marrow (n=5), peripheral blood (n=1), and both (n=1). Results. With a median follow-up duration of 13 months (range, 2~20), 1 patient died early due to severe thrombotic thrombocytopenic purpura and multi-organ failure. Another 1 patient died 7 months post-transplant due to pneumonia with sepsis. However, according to every 5 month-period monitoring of minimal residual disease by using real-time PCR method, the other 3 patients have been in excellent condition and all of them showed undetectable level of their BCR-ABL/ABL ratios without addition of imatinib after HSCT. All of them showed grade II of acute GvHD and progressed to limited type of acute GvHD without addition of imatinib after HSCT. All of them showed grade II of acute GvHD and progressed to limited type of acute GvHD, but they responded well to conventional treatment modality. Conclusions. In comparison to old control data, first-line imatinib interim therapy appears to provide a good quality of CR and a survival advantage for patients with Ph+ AML after allogeneic HSCT. Further long-term follow-up with large sample numbers is needed to validate the results of this study.

PROGNOSTIC SIGNIFICANCE OF FLT3 MUTATIONS IN DIFFERENT SUBTYPES OF ACUTE MYELOID LEUKEMIA

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Backgrounds. Fms-like tyrosine kinase 3 (FLT3) is a member of the class III receptor tyrosine kinase family along with KIT, FMS and platelet-derived growth factor receptor. Wild type FLT3 is expressed at high levels on 70% to 100% of blasts in acute myeloid leukemia (AML). FLT3 gene alterations, internal tandem duplications (ITDs) and Aspartate835 (DS835) mutations occur in 15%-30% in AML and may adversely affect clinical outcome. Aims. The aim of our study was to analyze the impact of FLT3 mutations in cohort of 113 newly diagnosed patients with AML on prognosis. Methods. Genomic DNA polymerase chain reaction (PCR) assay was performed to detect FLT3/ITDs located from exon 14 to exon 15 and we used PCR-restriction fragment length polymorphism (PCRRFLP) for detection of DS835 mutations in exon 20. Results. FLT3/ITD were detected in 20/113 patients (pt) (17.6%), DS835 mutations in 4/113 (3.5%) and both type of mutations in 1 (0.8%) pt. In the study group of 113 pts according to FAB classification, FLT3 mutations were found in all subtypes except M1, M6 and M7. The distribution of FLT3 mutations was as follows: FLT3/ITD were detected in M0 6/12 (50%) pts, 4/22 (16.7%) in M2, 3/14 (21.42%) M4, 3/24 (12.50%) M4, in 4/19 M5 pts (40.3%). DS835 mutation was found in 1 pt with M2 and M5 and 2 pts with M4 type of AML. Of 24 pts with FLT3 mutations a normal karyotype was found in 9 pts, 3 pts had translocation (15; 17), 2 pts had inv (16), one deletion of 19. Three pts had complex karyotype, and in 2 pts there were no karyotypes on preparation. Treatment included induction chemotherapy with doxorubicin 50 mg/m3 3 days and ara-c (200 mg/m3) in continuous infusion for 7 days. Consolidation therapy consisted of the same scheme or ADE combination. Complete remission in the whole cohort of patients was achieved in 62% and in
only 7/24 (29%) pts with FLT3 mutations (one patient with M0 and normal karyotype, one with M6a and also normal karyotype, 3 pts with M8 and translocation (15;17), one patient with M2 and deletion 19, one with M4 and inv(16). FLT3/ITD+ pts had significantly higher WBC count at diagnosis (WBC count for FLT3/ITD+ was 73.5±10/L and WBC count for FLT3/ITD- was 14.9±10/L, p<0.05). Median of overall survival of the whole group of pts was 11 months, and median of survival of pts with FLT3 mutations was 6 months. Conclusion: In contrast to other reports incidence of FLT3/ITD and D835 mutations are lower in our cohort although the study group was small and performed in a single Institution. With a median follow up of 46 months remission duration and overall survival were significantly shorter for patients with FLT3 mutations.

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MONITORING OF CARDIOTOXICITY DURING INDUCTION CHEMOTHERAPY CONTAINING IDARUBICIN IN ACUTE MYELOID LEUKEMIA WITH CIRCULATING MARKERS

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Backgrounds. Cardiac toxicity is a well-known and serious complication of antitumorous treatment. Anthracyclines represent the greatest risk for development of cardiotoxicity. Compts respond to the circulatory failure of structural and functional myocardial damage have been gaining ground in cardiotoxicity diagnostics. Aims. Monitoring of cardiotoxicity during induction chemotherapy in acute myeloid leukemia (AML) patients and assessment of the potential for use of circulating markers in early diagnostics of cardiotoxicity. Methods. Fifteen consecutive adult patients with a newly diagnosed AML (9 male and 6 female, mean age 43.7±10.6 years) participated in the study. The patients received induction chemotherapy containing intermediate doses of cytarabine and idarubicin (IDA) 12 mg/m²/day intravenously on day 1, 5 and 9 (in total 36 mg/m² = 1/4 of the maximum recommended cumulative dose). From circulating markers of myocardial damage we used a marker of cardiac dysfunction and failure - N-terminal pro brain natriuretic peptide (NT-proBNP), and two markers of myocardial necrosis (cardio-specific markers) - cardiac troponin T (cTnT) and creatine kinase MB (CK-MB mass). Serial measurements of plasma NT-proBNP concentrations were performed at the baseline, the following each IDA infusion, after 14 days and after circa 1 month, i.e. before the next chemotherapy. Cardio-specific markers (cTnT, CK-MB mass) were measured at the baseline and after the last IDA infusion. Results. The mean baseline concentration of NT-proBNP in newly diagnosed AML patients was 129.7±59.6 pg/mL. The mean NT-proBNP concentration increased after the first IDA infusion to 307.3±171.4 pg/mL (p=0.02). In most of the patients, the second and the third IDA infusions were not associated with a further increase in the NT-proBNP value and values after 2 or 4 weeks were not significantly different from the baseline. However, in one of the patients the NT-proBNP values were increasing after each IDA infusion (after the last one 796.2 pg/mL) and within 14 days he developed congestive heart failure due to left ventricular diastolic dysfunction as assessed by echocardiography. At that time, the NT-proBNP value was 11840 pg/mL after diuretics it decreased significantly. In all patients, plasma cTnT and CK-MB mass concentrations were within the reference interval at the baseline and after the induction chemotherapy. Conclusions. Our results show that induction chemotherapy in AML (IDA 36 mg/m² and intermediate doses of cytarabine): 1. does not cause detectable damage of cardiomyocyte structure, 2. is in all patients associated with acute neurohumoral activation (transient elevation of NT-proBNP) indicating acute subclinical cardiotoxicity, 3. may lead to congestive heart failure and NT-proBNP seems to be a promising early marker and predictor of this complication.

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WT-1, BCL2 AND BAX EXPRESSION AND CLINICAL OUTCOME IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background and aims. We prospectively evaluated the impact of WT-1, BCL2 and BAX expression on the clinical history of patients with non M3 AML and present here our preliminary results. Patients and methods. Forty patients have been included in the study. Complete molecular data are at the present available of 15 patients. Median age was 55 years (range 24-78); the cytogenetic evaluation at diagnosis (14 patients) disclosed a normal karyotype in 9 patients, a complex karyotype in 3 and other abnormalities in 2. All patients received as induction therapy fludarabine, Ara-C, idarubicin plus etoposide; 18 achieved CR (3 after second line therapy), 2 were refractory. All patients in CR received consolidation chemotherapy (13) or were submitted to autologous or allogeneic stem cell transplant. CR lasted a median of 14.5 months (range 3-48). Five patients have relapsed after a median of 8 months (range 3-17). At this moment 10 patients are alive and disease free in first or second CR. Median survival for the whole series of patients is 18 months (range 8-50). By real time PCR we studied the expression of WT-1, BCL2 and BAX on marrow samples collected at diagnosis and we evaluated the response to induction and every 6 months, in the follow up and tried to correlate molecular data with clinical outcome. Results. We classified patients in 3 groups according to the expression at diagnosis of WT-1 and BCL2. The 3 patients with absent or low expression of WT-1 and BCL2 at diagnosis maintain CR at 28, 30 and 48 months. Four out of the 5 patients with high expression of WT-1 or BCL2 at diagnosis achieved CR; 1 did not respond to induction therapy. Among the responsive patients 1 has died in CR after 17 months for transplant related complications, 2 have relapsed (at 11 and 14 months from diagnosis) and 1 maintains CR. Of the 3 patients with high expression of both WT-1 and BCL2 at diagnosis 1 did not respond to therapy, 2 achieved CR (in one patient lasted 7 months, in the second still ongoing at 12 months). Whereas the expression of BAX at diagnosis doesn’t seem to correlate with outcome, the level of BCL2 expression may have a relevant prognostic value. The 3 patients maintaining the first CR at 26-46 months had low levels at BCL2 expression at diagnosis. On the contrary patients with high levels of BCL2 had a poor outcome: one did not respond to therapy, other two showed a chemorefractory relapse at 7 and 15 months from diagnosis. The longitudinal evaluation of BAX-BCL2 ratio may give information on the relapse risk, with the progressive reduction of this ratio indicating impending BAX-BCL2 ratio may precede of some months the increase of WT1. Conclusion. These preliminary results indicate that molecular evaluation at diagnosis of WT-1, BCL2 and BAX might have prognostic value and that prospective comparative evaluation of BAX-BCL2 ratio might predict relapse.

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PROGNOSTIC RELEVANCE OF SOLUBLE TPO LEVEL IN AML

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Backgrounds. Thrombopoietin (TPO), the major growth factor for cells of the megakaryocytic lineage is removed from circulation by binding to c-mpl receptors present on platelets and megakaryocytes. Recent studies revealing c-mpl receptors was reported on the AML blast cells and its clinical impact on AML prognosis remains to be characterized. Aim. Is to determine the level of TPO in AML patients in order to characterize its clinical relevance. Methods. We assessed TPO levels by ELISA in 41 AML patients at diagnosis, after 28 days of induction chemotherapy and at AML remission. Follow up for the patients was done up to 24 months. Results. TPO levels was significantly higher at diagnosis as compared to normal controls (p<0.01). At 28 days after induction chemotherapy the TPO level continue to elevate and was significantly higher as compared to the diagnosis level (p<0.01), and then decline during remission reaching near the control level (p=0.05). The TPO levels was inversely correlated to the platelets counts (R=0.9 , p<0.01). TPO level at AML diagnosis was significantly lower in a group of patients who died during the follow up course(n= 25) and in patients resist induction chemotherapy (n=8) as compared to patients who survive and patients who respond to chemotherapy (p<0.05 for both).

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IMPACT OF ADDITIONAL CYTOSGENIC ABNORMALITITIES ON REMISSION INDUCTION RATE EVENT FREE AND OVERALL SURVIVAL IN 34 PATIENTS WITH NEWLY DIAGNOSED ACUTE PROMYELOCYTIC LEUKEMIA TREATED WITH APSL3.TUNISIAN EXPERIENCE

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Additional chromosomal abnormalities in acute promyelocytic leukemia (APL) are associated with an increased risk of relapse and death. Recently, we have developed a new induction chemotherapy protocol (APSL3) for newly diagnosed APL patients in which ATRA is not used as the induction treatment. We report the outcome of 34 APL patients treated with APSL3.
leukemia (AML) are observed in around 30% of cases. Their presence seems to have an impact on prognosis. Our aim in this study is to analyse impact of additional cytogenetic abnormalities on complete remission rate (CCR), event free survival (EFS) and overall survival (OS) in 34 consecutives patients with APL and t(15;17) treated with APL93 protocol between 1998 and 2004. Median age was 28 yr (6-60 yr). Median WBC was 8000/mm³ (600-97000/mm³). Informative karyotype was obtained in all patients. Additional cytogenetic abnormalities were seen in 9 patients 26.47% (9/34). These abnormalities were: +8(4), add 9q(1),del 9q12;q31(1), del 17q(1),+15p11(1),add 5p15(1). For all patients CRR was 82% (28/34), failure of induction was due to 6 toxic deaths: sepsis (1), ATRA syndrome (2), SNC hemorrhage (2), and diabetes (1). EFS at 4 yr is 63, 47% and OS at 4 yr is 69, 72%. Outcome was similar between patients with t(15;17) alone and patients with additional cytogenetic abnormalities for CCR 84% (21/25) vs 77.7% (7/9) p=0.6, for EFS at 4 yr -62.02% vs 66.67% p=0.74 and for OS at 4 yr 64.81% vs 70.82% p=0.5. Our study does not find any significant impact of additional abnormalities despite a little advantage for EFS and OS for this group.

HUMORAL IMMUNE RESPONSE AGAINST THE PRAME ANTIGEN IN PATIENTS WITH MYELOID LEUKEMIAS

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Backgrounds. The PRAME (preferentially expressed antigen in melanoma) is expressed at high levels in various malignant tumors including hematopoietic malignancies, especially in acute myeloid and lymphoid leukemias (AML, ALL), multiple myeloma. It has no or weak expression in normal tissues making it a candidate for immunotherapy. PRAME can also elicit T-cell immune response in melanoma patients but there are no data concerning anti-PRAME immune response in leukemias. Aims. To detect specific immune response towards PRAME in patients with myeloid leukemias (AML, CML). Methods. Sera obtained from patients with myeloid leukemias were analyzed in enzyme-linked immunosorbent assay (ELISA) for detection anti-PRAME antibodies. Results. IgG PRAME antibodies were measured in 122 patients (25 AML, 97 CML) and 22 healthy volunteers. Immunoglobulin IgG PRAME antibodies were detected in 4 (16%) and 8 (8%), respectively, of 122 patients, whereas none of the healthy volunteers had IgG PRAME antibodies. In one of IgG PRAME ‘positive’ AML samples the specific cytotoxic T-lymphocytes were founded by MHC-peptide tetramer staining with intracellular interferon-γ co-staining. Summary: The data demonstrate that spontaneous humoral immune response against PRAME protein could be detected in the patients with PRAME-expressing hematopoietic malignancies.

IS LEUKEMIC SUFERSABLE TO IMPROVE SURVIVAL IN HYPERLEUKOCYTIC AML IF USED AS THE EARLY CYTOREDUCTION TREATMENT?

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Background. The management of patients with AML presenting with hyperleukocytosis remains controversial. In spite of relatively high incidence of hyperleukocytic AML (7-15%) and its very high early mortality (>40%), there is no consensus in the optimal treatment for a prompt leukoreduction. Aims. The aim of this retrospective non-randomized study was to compare early mortality and overall survival (OS) in patients with hyperleukocytic AML initially treated with either hydroxyurea (HU) alone or HU and leukapheresis. Patients and Methods. From 1998 to 2005, 40 patients were treated for hyperleukocytic AML (MO-2, M1=8, M2=7, M3=2, M4=16, M5=5) in our institution. Group A consisted of 20 patients with median age of 67 years (19-78) treated by HU only (50 mg/kg/day in 3 or 4 daily doses). Group B consisted also of 20 patients with median age of 53 years (19-72) treated with HU and cytoreduction leukapheresis. The intention of the cytoreduction treatment was to reduce WBC count to at least 50×10⁹/L before administration of an induction chemotherapy to prevent complications from leukostasis and tumor-lysis syndrome. Leukaphereses were performed using COBE Spectra cell separator. Results. The early mortality was high according to the expectations: seven patients died within two weeks in group A, as well as in group B. The patients from the group B were generally in worse condition and 4 of them died within the first 48 hours for intracranial hemorrhage or respiratory failure (ARDS) because of leukostasis. To target cytoreduction in the group A was delayed compared to the group B, although the initial WBC count was lower (160×10⁹/L vs. 200×10⁹/L, means). In the group B, forty leukaphereses were performed in total, median 2 (1-4) per patient. Induction chemotherapies could started earlier in the group B compared to group A: on day 4 (median, range 2-12), median WBC count 30.2×10⁹/L and on day 7 (median, range 3-14), median WBC count 24×10⁹/L. Thirty induction chemotherapies were administered in total, 14 in the group A and 16 in the group B. One patient from the group B refused chemotherapy and died of leukemia in 11 days. Complete remissions were reached in 15 patients, but only in 5 from the group A. OS was significantly longer in the leukopheresis group (p<0.05), however, we did not confirm improvement of the 2-week mortality. Median OS in the group A was 30 days and no patient survived more than 500 days. Median OS in the group B is 282 days and 6 patients are still alive from 2 to 5.7 years after the AML diagnosis. Summary/conclusions. Current published data do not define the impact of using leukapheresis for the cytoreduction before the individual response in a clinically relevant time, we analysed the clearance of peripheral blasts (PBC) in 30 AML patients during “4-7” induction course. Methods. By extensive flow cytometry (FC), a population of cells with leukaemia-associated aberrant immunophenotype (LAIP) was identified in each patient from the initial bone marrow (BM) aspirate. We then obtained the percentage of positive counts on peripheral blood (PB) immediately before starting therapy (day 1) and every day until day 8. PBC was expressed as the ratio, converted to logarithmic scale, between baseline value (day 1) and daily absolute blasts count. At day 14, FC analysis was performed on BM in order to identify LAIP-positive residual blasts. The degree of BM clearance was expressed as the ratio, converted to logarithmic scale, between the percentage of LAIP-positive blasts determined at diagnosis and day 14 (LD14). Results. Between May 2004 and January 2006, 30 consecutive newly diagnosed non-M3 AML patients aged less than 66 years entered the study and were evaluable for BM response. After a single course, complete remission (CR) was achieved in 17 patients. CR was not obtained in 13 patients (NCR), 8 of whom were refractory. According to conventional criteria (cytogenetics and secondariness) there were 11 high risk patients, of whom 4 achieved CR; 14 intermediate risk patients, of whom 8 achieved CR; 5 low risk patients, all of whom achieved CR. The ranges of distribution of PBC had minimal overlap between CR and NCR groups. Since in patients who achieved CR, by day 7 or 8 blasts were often already undetectable, we excluded these time-points from analysis (Figure 1A). The medians of log reduction in the two groups were significantly different on each day (Figure 1B). The rate of PBC appeared higher in CR than NCR patients with an estimated difference between groups equal to 0.26 (95% CI: 0.15-0.37, p value<0.001). This difference was not attributable to differences in baseline PBC leukemic burden and assigned risk. PBC showed an excellent correlation with BM response as assessed by morphologic analysis at haematopoietic recovery and by FC on day 14. Specifically CR was not achieved in any of 11 patients who had a PBC below 2 logs on day 5, whereas CR took place in 17 out of 19 patients who had a PBC greater than 2 logs on day 5. Higher values of PBC on each day were associated with larger LD14 (Figure 1C). This correlation was significant on each day and it increased monotonically over days. Summary/conclusions.
These data indicate that PB may be in equilibrium with BM in each AML patient, and that PB clearance gives evidence of BM clearance. Therefore, a major treatment outcome may be predicted very early during the induction therapy of AML patients, thus providing an opportunity to tailor treatment modalities since the outset.

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PROLIFERATION OR APOPTOSIS INDUCED BY HALOFGUNITONE IN ACUTE PROMYELOCYTIC LEUKEMIA CELLS DEPEND ON THE INTENSITY OF TGFβ INHIBITION  
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The Transforming Growth Factor-β (TGF-β) is a multifunctional cytokine that plays an important role in cellular homeostasis by regulating cell growth inhibition, differentiation, cellular senescence and apoptosis. A key regulator of TGF-β function is the cytoplasmic isoform of the PML protein. In fact, the inactivation of the PML gene has been demonstrated to cause resistance to TGF-β-dependent growth arrest, induction of cellular senescence and apoptosis. The PML-RARα fusion protein, generated by t(15;17) in acute promyelocytic leukemia (APL), exerts a dominant negative action on PML and thus indirectly leads to deregulation of TGFβ pathway. However, the cross talk between PML and TGFβ pathways in APL has been poorly characterized. To address this issue, we have analyzed the effect of progressive inhibition of TGF-β activation by Halofuginone (HF), a low molecular weight quinazolino-none alkaloid in NB4 cell line. Using a four color flow cytometric method, we simultaneously analyzed BrdU incorporation, cell cycle status, apoptosis and Bcl-2 expression in NB4 cells incubated for 24 hours with increasing doses of HF (6.25, 12.5, 25 and 50 ng/ml). An increase in the percentage of cells in S-Phase was observed with low concentrations of HF (lower than 12.5 ng/ml), whereas higher doses induced apoptosis blocked cell cycle at G2/M and inhibited Bcl-2 expression. These results were confirmed by a separated set of experiments in which apoptosis was evaluated by annexin V and propidium iodide labelling and cell proliferation was evaluated by trypan blue exclusion. TGF-β activation was evaluated by determination of its downstream effector Smad 4 levels. These results indicate a bimodal pattern for the effect of TGFβ inhibition in APL. Decrease, but not abrogation, of the activation of this pathway has a proliferative effect, probably by facilitating PML-RARα dominant negative action on cytoplasmic PML. In contrast, complete block of TGFβ signaling leads to apoptosis associated with Bcl-2 inhibition.

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CLINICAL SIGNIFICANCE OF MULTIDRUG RESISTANCE 1 (MDR1) GENE EXPRESSION FOR TREATMENT OUTCOME IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA  
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A major cause for early relapse and treatment failure in patients with acute lymphoblastic leukemia (ALL) is the occurrence of multidrug resistance (MDR). One of the mechanisms is the overexpression of MDR1 gene which encodes a drug efflux pump called permeability-glycoprotein (P-gp). The aim of this prospective study was to analyse the expression of MDR1 gene and correlate our findings with clinical, laboratory parameters and treatment outcome in children with ALL. Material and Methods. We studied prospectively 49 children with ALL (26 boys and 23 girls) with a median age of 5.1 years (range: 15 months to 13.9 years). Four children were also evaluated at initial diagnosis and relapse. All patients were treated according to the BFM95 chemotherapy protocol with a median observation time of 18 months (range 9-36 months). As controls we used bone marrow (BM) mononuclear cells from 7 children who underwent a BM biopsy for diagnostic purposes and was negative for leukemia. Total RNA was isolated from BM samples at initial diagnosis and relapse. The expression of the MDR1 gene and the housekeeping β-actin gene was detected by RT-PCR using the appropriate primers. After electrophoresis of the PCR products in 1.5% agarose gel stained with ethidium bromide, gels were scanned by UV transillumination with a densitometer. The relative mRNA expression of MDR1 gene was calculated using the following formula:

Expression Index (EI): MDR1 PCR product / β-actin PCR product

Results. The mean MDR1 gene expression was significantly higher compared to the control group (p<0.05). The MDR1 gene expression in patient samples ranged from 0.02 to 2.49 (median 0.35). Using the median as a cut-off value for high and low expression, high MDR1 gene expression was found in 18 (36.7%) patients and their event free survival was significantly worse compared with children with low MDR1 expression (86.67% vs. 55.56%; p log-rank: 0.03). High expression of MDR1 gene did not correlate with immunophenotype, NCI risk classification, white blood count, prednisone response on day 8, and LDH value. Interestingly, significantly higher MDR1 expression was found at relapse in four paired samples compared with diagnosis. Cox regression analysis revealed that children with high MDR1 expression at diagnosis had a relative risk of 3.36 (range: 1.02-11.46) for failure to achieve a complete remission or relapse (p=0.04). Conclusion. The expression of the MDR1 gene in childhood ALL is a useful tool in assessing the risk of treatment failure and early relapse and can be used as an additional prognostic factor.

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PERIPHERAL BLOOD B-LYMPHOCYTE SUBSETS AFTER TREATMENT OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA  
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The immunosuppressive effect of cytotoxic drugs, basic therapeutic agents in the treatment of childhood acute leukemias, requires monitoring of the immune system following cessation of therapy. The aim of the study was to examine the relation of the relative and absolute numbers of CD19+, CD23+ and CD5+ B lymphocytes in peripheral blood in children with acute lymphoblastic leukemia (ALL) after cessation of chemotherapy. The examined group included 150 children with standard-risk ALL treated with standard chemotherapy protocol. The analyses were performed directly after therapy in 30 children, in 30 children - 3 months later, in 30 children - 6 months later, in 30 children - 9 months later, and in 30 children - 12 months after therapy cessation. The control group consisted of 30 healthy age-matched children. Lymphocyte populations were analyzed by multiparameter flow cytometry with 3-color analyses. Phenotypes of B-cell subsets were obtained with anti-CD19 antibody plus combinations of FITC and PE-labeled antibodies specific for CD5, CD23. The data were acquired and analyzed by Cell Quest software (Becton Dickinson). The proportion of lymphocytes stained with each monoclonal antibody was converted to the absolute per microliter by multiplying the absolute number of lymphocytes per
A TOXIC EFFECT OF METHOTREXATE ON THE BRAIN OF CHILDREN WITH ACUTE LYMPHOLASTIC LEUKEMIA

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In treating acute lymphoblastic leucosis (ALL) in children in terms of ALL-BFM-90(m) program, methotrexate (MTX) utilized intravenously, 1 g/m², combined with an intrathecal injection penetrates the hematoencephalic barrier and harms the brain. Purpose. Disclosure of early adverse brain reactions. A bioelectric brain activity (BEAB) was studied using electromyogram (EEG) Nihon Kohden, Japan. Fecundities of the brain blood supply were investigated utilizing transcranial ultrasound dopplerography (TUSDG) Log 104, Kränzbuhler, Germany. Quantitative assessment of the EEG values was done through the program of carting the DX-complexes-4000, including spectral analysis on the basis of Fourier transformation. The results showed that the relative expressions of GR-α and GR-β in newly diagnosed and relapsed childhood ALL cases treated in a tertiary hospital in Malaysia is still unknown. Aims. The aim of this study was to determine the relative expression pattern of glucocorticoid receptor (GR) isoform mRNA in newly diagnosed and relapsed childhood ALL cases treated in a tertiary hospital in Malaysia. Methods. Blasts were isolated from total of 13 cases of childhood ALL, of which 6 were at initial diagnosis, 6 at relapse and one case with paired samples at both diagnosis and relapse. Total RNA was extracted from cell pellets of leukemic blasts. The relative mRNA expression of GR-α and GR-β was determined by quantitative RT-PCR using fluorogenic Syber Green 1. The quantitative value is reported as 2-DDct, which gives the mean fold change in gene expression normalized to GAPDH as an endogenous control, and relative to the mRNA expression of blast from non-malignant disorders in children. Results. GR-α mRNA expression was not significantly different between newly diagnosed and relapsed children. The GR-α/GR-β ratio in newly diagnosed ALL were 0.94 and 0.32 fold respectively, and for relapse ALL were 0.36 and 0.16 fold respectively. The GR-α/GR-β ratio was higher in the newly diagnosed ALL group (2.95) while the ratio was lower in the relapsed cases (2.25). In the only case of ALL where paired samples were analyzed, the GR-α/GR-β ratio was also decreased at diagnosis compared to at diagnosis. The GR-α and GR-β are expressed in our acute leukemia cases. The GR-α/GR-β ratio in newly diagnosed ALL was lower in our relapsed ALL group compared to the newly diagnosed ALL cases, as previously reported. However, further studies must be performed in a larger group of ALL cases, to correlate the glucocorticoid response with these GR isoforms expressions.

RELATIVE QUANTIFICATION OF GLUCOCORTICOID RECEPTOR ISOFORMS MRNA IN CHILDHOOD ACUTE LYMPHOLASTIC LEUKEMIA (ALL) A PRELIMINARY REPORT OF 13 CASES

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Backgrounds. Acute lymphoblastic leukemia (ALL) is one of the commonest cancers in children. Combination chemotherapy remains the first line of treatment for ALL and glucocorticoids such as prednisolone and dexamethasone are amongst the key drugs in the regime. The effects of glucocorticoids are mediated by the binding and activation of their intracellular receptor (GR), a member of steroid receptor superfamily. The ability to respond to the treatment is determined by concentration of GR per cell. Alternative splicing of glucocorticoid receptor (GR) gene results in several isoforms namely the GR-α, GR-β, GR-γ and GR-P. The quantity of the GR isoforms mRNA in leukemic blasts has been reported to be correlated to the ALL phenotype and also their sensitivity to glucocorticoids. GR-α is a functional intracellular receptor that cannot bind glucocorticoid and may have a dominant negative effect on GR-α. Reports have shown that GR-α/GR-β ratio is decreased in resistant patients. However the association of the expression pattern of GR isoforms with their sensitivity to glucocorticoids in childhood ALL in Malaysia is still unknown. Aims. The aim of this study was to determine the relative expression pattern of glucocorticoid receptor (GR) isoform mRNA in newly diagnosed and relapsed childhood ALL cases treated in a tertiary hospital in Malaysia. Methods. Blasts were isolated from total of 13 cases of childhood ALL, of which 6 were at initial diagnosis, 6 at relapse and one case with paired samples at both diagnosis and relapse. Total RNA was extracted from cell pellets of leukemic blasts. The relative mRNA expression of GR-α and GR-β was determined by quantitative RT-PCR using fluorogenic Syber Green 1. The quantitative value is reported as 2-DDct, which gives the mean fold change in gene expression normalized to GAPDH as an endogenous control, and relative to the mRNA expression of blast from non-malignant disorders in children. Results. GR-α mRNA expression was not significantly different between newly diagnosed and relapsed children. The GR-α/GR-β ratio in newly diagnosed ALL were 0.94 and 0.32 fold respectively, and for relapse ALL were 0.36 and 0.16 fold respectively. The GR-α/GR-β ratio was higher in the newly diagnosed ALL group (2.95) while the ratio was lower in the relapsed cases (2.25). In the only case of ALL where paired samples were analyzed, the GR-α/GR-β ratio was also decreased at diagnosis compared to at diagnosis. The GR-α and GR-β are expressed in our acute leukemia cases. The GR-α/GR-β ratio in newly diagnosed ALL was lower in our relapsed ALL group compared to the newly diagnosed ALL cases, as previously reported. However, further studies must be performed in a larger group of ALL cases, to correlate the glucocorticoid response with these GR isoforms expressions.
counts, a BM aspiration demonstrated an ALL relapse. Systemic and its chemotherapy was administered, however without any improvement of hearing or vision loss. The patient died of septicemia three weeks after admission. Discussion. The first case illustrates that bilateral hearing impairment may represent the sole symptom of CNS relapse of ALL. The ophthalmic significance of the patient’s hearing problems was overlooked. The patient responded well to it therapy, with improved hearing, and BM disease did not follow CNS relapse. Apparently, immunosuppression, albeit not protecting the patient against CNS relapse, did prevent fulminating hematological relapse. Also in the second case, there was a substantial doctor’s delay due to patient’s initially seemingly harmless symptoms in combination with normal blood counts. In both cases hearing impairment was caused by neuropathy in the auditory nerve. We conclude that hearing impairment in an ALL patient, even if slow, bilateral, and isolated, should raise the suspicion of CNS disease.

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**HEMATOGONES (B-CELL PRECURSORS) AFTER CHEMOTHERAPY IN PATIENTS WITH ACUTE LEUKEMIA BY 3-COLOR FLOW CYTOMETRY**

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Backgrounds. Hematogones, B-cell precursors are present in small numbers in the bone marrow and peripheral blood. The immature mononuclear cells including hematogones in the bone marrow aspirates should be differentiated from leukemic blasts in the post-chemotherapeutic bone marrow aspirates and patients with acute leukemia. Annually, the patterns of hematogones were evaluated to differentiate them from residual leukemic blasts in the post-chemotherapeutic bone marrow aspirates of patients with acute leukemia. Methods. The bone marrow aspirates (10 hypocellular marrows, 11 complete remission states, 2 persistence states of acute leukemia, 2 post-BMT states) from 25 cases of acute leukemia (7 AML, 6 ALL) were included to measure hematogones by 3-color flow cytometry (CD19-FITC/CD10-PE/CD34-PerCP). Hematogones were defined as the mononuclear cells with coexpression of CD10 and CD19. We analyzed the patterns of hematogones and the correlation of the proportions of total hematogones & more immature CD34(+) hematogones with patients’ ages & the hematologic diagnosis of the bone marrow aspirates. Results. The groups of patients with less than 1% (N=11, group I), 1-5% (N=7, group II), & equal or more than 5% (N=7, group III) of hematogones in the bone marrow aspirates show 14.5 years, 10.1 years & 8.2 years of the mean ages each, and 6.8%, 43.2% & 48.4% of the mean proportions of CD34(+) hematogones. We could not find any differences of hematogone patterns between AML and ALL, but according to the post-chemotherapeutic bone marrow states the different findings were noted. In hypocellular marrows and in complete remission states, there were 74.0% & 22.1% of the immature or mature lymphocytes of hematogones among nucleated cells and 12.3% & 55.9% of hematogones among B-cells. Conclusions. By 3-color flow cytometry (CD19/CD10/CD34) hematogones could be differentiated from residual leukemic cells in the post-chemotherapeutic bone marrow aspirates of patients with acute leukemia. We found that the hematogones, especially more immature hematogones increase more in the younger patients and that the proportions of hematogones are lower in hypocellular marrows inspite of higher proportions of lymphocytes than in complete remission states.

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**LATE EFFECTS OF CHILDHOOD ALL TREATMENT ON GONADAL FUNCTION IN MALE SURVIVORS**


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Current treatment protocols in ALL aim survival with minimal effect on fertility. However, gonadal function is at least finally affected irrespective of treatment age in males with ALL. The aim of our study was to evaluate gonadal function in our male survivors of childhood ALL in the context of pubertal stages. Subjects consisted of 55 males (≥9 years old) diagnosed between 1975 and 2002 in our department, after a follow up of 10.07±9.00 years. Twenty-nine healthy males of similar chronological age (CA) were taken as controls. Mean CA of the study (Group I=G I) and control (Group II =G II) groups were 15.65 ± 4.79 and 15.42 ± 5.73 years respectively (p<0.04). Forty six patients who had received RT (43; CRT, 2; CSRT) will be indicated as Group IA (GIA). Serum FSH, LH, estradiol (E2), total (T) and free testosterone (FT), inhibin-B, sex hormone binding globulin (SHBG), bilateral testicular ultrasound and semen analysis (in patients > 16 years old, N=24; controls N=9) were evaluated. Patients with the mean proportions of hematogones were classified as prepuberty (Tanner stage I=1), early (Tanner stage II–III=2) and late puberty (Tanner stage IV–V=3). Semen analysis was evaluated in the context of categorized sperm counts (azo/oligo/o-a normozoospermia) between GI and GI. Results. Inhibin-B was significantly lower in GIA than in GI in PC 1. Estradiol and FT were significantly higher and SHBG significantly lower in GI, LH than in GI in PC S. In conclusion, despite normal inhibin-B levels in early puberty and late puberty, low inhibin-B levels were found in prepuberty. Our findings suggest it might be due to CRT. High E2 and FT levels and appropriately low SHBG levels in late puberty indicate hormonal levels are not yet compromised in these individuals as also reflected by normal FSH, LH, and T levels. Testicular volumes were not reduced in prepuberty, early puberty and late puberty. Sperm counts were not significantly affected.

**1375**

**LYMPHOMAS IN AIDS**

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For the last 3 yrs we have followed 51 lymphoma - AIDS pts. 96% were drug users and 80% were association with HCV. Non Hodgkin’s lymphomas were diagnosed in 37 pts. Male- 23, female- 14, median age 30. CD4 counts were from 20 to 500 (median 300) cells cu/mm. Viral load was from 10000 to 500000 copies /ml. Histological diagnosis by biops sy and postmortem was received in all pts and immunohistochemistry was performed in half of them. Diagnostic laparotomy for lymph node biopsy was done in 6 cases, thoracotomy in 1, orchectomy in 1, splenectomy in 6 pts. The most often diagnosis to be differentiated from our cases was TBC, established in 40% of lymphadenopathy-AIDS pts Diffuse B- large cell lymphomas happened in 11, Burkitt lymphoma in 6, MAL- omas in 4, Fucicular lymphoma 1, Plasmoblastic lymphoma 1, MALTomas in 1, T-cell lymphoma 1, primary CNS Lymphoma in one pts. 16 pts had not received treatment and died soon after admission. 21 pts received CHOP (4 with daunoxome), blocks A-B-C of BMF- NHL- 95 with CNS prophylactics and Mabthera, ESHAP. Complete remissions were reached in 6 pts, died from lymphoma progression 10 pts, 5 pts are on therapy with good response. Hodgkin’s lymphoma was established in 14 pts. Male- 11, female- 3, median age 30. CD4 counts from 400 to 1500 cells cu/mm, VL from 1000 to 100000 copies /ml. Mixed cellular variant was established in most cases 8 pts had not received polychemotherapy because of late admittance and poorest performance status. Chemotherapy. 11 pts achieved complete remission: on COPP, ABVD, BEACOPP2. 3 pts achieved complete remission.1 pt died from TBC in complete remission. 6 pts are on therapy and on HAART with good response. Post treatment CD4 is 1000- 2000 cells cu/mm, VL 1000 to 5000 copies /ml. Extragonadal germ cell tumors were seen in 3 pts, one female. In one pt 1 yr remission was achieved on BEP therapy. HAART. Conclusion. HIV/AIDS pts with malignant lymphomas may receive diagnostic and treatment approaches which in results may be compared with general population. They must have opportunity to enter general hematological service all the country.

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**RAPID INFUSION OF RITUXIMAB CAN BE GIVEN SAFELY AND HAS A SIGNIFICANT IMPACT ON CAPACITY**

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Administration of Rituximab can be associated with infusion related toxicity. The risk is greatest with the first infusion and lower for subsequent infusions. To minimise the risk of reaction stict infusion guidelines have been developed involving lengthy infusion times. With the addition of steroids in combination with Rituximab the risk of reaction may be lower as the length of infusion time shorter. Since April 2005 16 patients in our institution, having RCVF and RCHOP for NHL, received Rituximab at the rapid infusion rate. The first infusion was delivered according to the product monograph. All subsequent cycles were given over a total infusion time of 90 minutes (20% of the total in first 30 minutes, the remaining 80% in 1 hour). The patients were closely monitored...
A RETROSPECTIVE ANALYSIS OF 57 CASES OF MANTLE-CELL LYMPHOMA ADMITTED IN THE CLINIC OF HEMATOLOGY- FUNDENI INSTITUTE BETWEEN 1994-2004

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Background. Mantle-cell lymphoma (MCL) represents a problem for the haematologist due both to the difficulty in establishing the diagnosis and to the lack of response to standard lymphomas protocols therapy. Aims. The aim of this study was to analyze the clinical aspects, the identification of major prognosis factors as well as the therapeutic results in 57 cases of MCL admitted in the Clinic of Haematology-Fundeni between 1994-2004. Methods. The diagnosis was based on the histologic examination (WHO criteria) and/or immunophenotyping tests (CD5+, CD23-). Results. Their median age was 61 years, M:F rates 1:2.51; 81% of patients presented in an advanced stage of disease (stage III-IV Ann Arbor) with generalized adenopathies (54%) and/or organomegaly (38%). The site of presentation was stomach (75%), with <2 ECOG. Bulky disease was detected in 26% and extranodal determinations ≥ 2 m in 84% of cases. Lymphocytosis ≥ 4,000 /µl in 81% and ≤ 10,000 /µl in 51% of cases, anemia (Hb < 12 g/dl) in 50% and bone-marrow involvement in 75% of cases. Other extranodal localizations were recorded in gastrointestinal tract (15%), liver (36%), pleura (17%), Waldenry ring (7%), skin (7%), orbital space (1%) and Nervous Central System (1%). LDH had increased values over normal limits in 64%. M-component in blood in 14%. 68% had an IPI score > 2 and 32% an IPI score ≥ 2. The initial therapy started with Chlorambucil+Prednison (16 cases); CVP/COP (21 cases) and CHOP or CHOP-like (21 cases). Fludarabine+Cyclophosphamide were introduced only in relapsing or refractory diseases in 9 cases. Rituximab was administrated in 3 cases (one administration per week) and R-CHOP-like was given in 1 case. Splenectomy was carried out in 3 cases. α interferon was applied in 4 cases (3 administration x 3 MU each per week) as a maintenance therapy. In 25 cases (40%) complete and partial remissions were obtained. The median survival time for the whole lot was 20 months (3-108 months). The univariate analysis revealed that good performance status (ECOG 1,2), limited clinical stages (I, II) no bulky disease, hemoglobin level >12 g/dl, normal values of LDH and IPI <2 were major predictive factors correlated with long survival. The prognosis was more favorable in cases with large cell lymphom (35%) whereas the non-Hodgkin lymphomas represented a problem of diagnosis and therapy; the evolution is ineluctable fatal as the disease is largely disseminated at presentation, is generally resistant to standard therapy or localized radiotherapy. One patient has been follow-up alive without treatment because he didn’t accept treatment. All the treated patients achieved complete remission (95%) except one who achieved partial remission. Summary/Conclusions. Because of the indolent course the prognosis of MALT lymphomas was good regardless of the treatment modalities used, and MALT lymphomas is a clearly defined. The treatment choice should be patient-tailored, taking into account the size, stage, grade and other clinical characteristic of patient.

EVALUATION OF PERIPHERAL BLOOD AND BONE MARROW INVOLVEMENT IN MANTLE CELL LYMPHOMA (MCL) - IMMUNOPHENOTYPIC AND MORPHOLOGICAL FINDINGS

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MCL is a B-cell malignancy with distinct molecular genetics and pathological features. It has been reported that advanced Ann-Arbor stage IV and especially leukemic phase of the disease are associated with poorer prognosis. This study aimed to evaluate immunophenotypic and morphological features of the lymphoma cells in bone marrow (BM) and/or peripheral blood (PB) in MCL patients (pts) with clinical stage (CS) IV and V (leukemic phase) in comparing with morphological and clinical features. Forty pts were studied (med. age 62, range 57-82 y., M/F=1.93:1) from 1996-2005. Overall survival (OS) for all pts was 21.5±18.7 [4-82] months. CS IV and V pts had the leukemic phase of the disease. Presenting features included splenomegaly (75%), hepatomegaly (19%), lymphadenopathy (50%), gastro-intestinal and sinus involvement (6%), respectively. Mean hemoglobin (Hb) was 11±4 g/dl, platelets 131±9×10^9/l, and leukocytes 51±66×10^9/l. The pattern of marrow biopsy involvement was diffuse (47.5% pts), interstitial (30% pts), nodular (15% pts) and paratrabeicular (7.5% pts). Morphological blastoid variant of MCL was found in 7 (17.5%) pts, whereas the rest of pts had small cells and standard MCL cells morphology. Immunophehnotyping and multiparameter flow cytometry were done on the PB (87.5%) or BM (12.5%) samples. Surface markers were identified using monoclonal antibodies against CD19, CD20, CD22, CD10, FMC7, CD5, CD23, CD38, CD79b, CD2, CD8, kappa, and lambda antigens (Ag). Immunologcal markers showed a typical expression pattern in all patients: CD19+, CD20+, CD5+, CD22+, CD1, CD20, CD4, CD8, CD3, kappa, and lambda antigens (Ag). Immunologcal markers showed a typical expression pattern in all patients: CD19+, CD20+, CD5+, Cyclin D1+, CD38+. Spearmain pairwise positive correlations between expression level of CD19 and CD20, CD20, CD8, CD79b Ags were found (<p<0.05). There was a strong positive correlation between expression level of CD38 and CD79b Ags (<p<0.05) as well as between CD20 and CD79b Ags (<p<0.05). Presence of blastoid variant of MCL was associated with higher proportion of cells with CD23 Ag expression, comparing to pts who hadn’t blastoid variant of the disease (22.5% vs. 9.5%). Wald-Wolfowitz z = 5.926, p<0.01. There was a significant predominance of positivity for following Ags: CD22 (73.5% pts), FMC7 (76.19% pts), CD79b (94.4% pts), and CD38 (86.7% pts) among the whole group of pts (Hi square test, p<0.05). The results of the current study demonstrated that among the whole group of pts with CDS-
CD23- phenotype, pts with blastoid variant had significantly higher proportion of cells with CD25 expression, despite of pts with standard cytomas. Differences in immunophenotype between pts with blastoid variant and small cells or typical MCL cells, desire prospective analyses in large cohort of pts, and give some insights about their biological features.

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IS GASTRECTOMY NECESSARY FOR NON-HODGKIN WITH GASTRIC INVATION?
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CHOP or R-CHOP has been established as a standard first-line chemotherapy for non-Hodgkin lymphoma (NHL). On the other hand, gastrectomy with postoperative chemotherapy for NHL of stomach has been considered as a standard therapy same as for gastric cancer. Recently, there are some reports that chemotherapy and radiation therapy (RT) for NHL of stomach has same or more survival rate compared with gastrectomy. In this study, we investigated the utility of chemotherapy and the necessity of gastrectomy for the NHL patients with gastric invasion From 1998 January to 2005 October, 91 NHL patients were admitted to our hospital. With endoscopic examination, they were grouped in NHL groups with gastric invasion (GI) (n=15) and without gastric invasion (NGI) (n=76). The average of GI age was 65±10, and NGI was 62±15. Gender (M:F) was GI (7:6) and NGI (44:34). PS(0,1,>2) was GI(9:4) and NGI(46:32). According to Ann Arbor classification, Grade I and II were defined as a mild group, Grade III and IV were defined a severe group. Seventy (mild or severe) was GI (21:11) and NGI (26:52). Pathological diagnosis of DLBCL was 10 cases (77%) in GI, 54 cases (69%). Average of blood Hb was 11.7±1.1 g/dl in GI and 11.8±1.8 in NGI. Average of albumin was 3.6±0.6 in GI and 3.5±0.6 in NGI. CHOP and R-CHOP were given in 6 cases and 7 cases of GI, 69 cases and 9 cases of NGI. There were no differences between GI and NGI in background of patients. There was significantly no difference cumulative survival rate in between GI and NGI. Also, in GI group, there was no difference cumulative survival rate in between mild group and severe group. It was suggested that chemotherapy of CHOP or R-CHOP is effective for NHL patients, whether there was gastric invasion or not. Gastrectomy might not be necessary for NHL patients except emergency and special cases.

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CEOP RITUXIMAB: AN EFFECTIVE AND SAFE REGIMEN FOR ELDERLY PATIENTS > 75 YEARS OLD WITH DIFFUSE LARGE B-CELL LYMPHOMA
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Backgrounds. About one quarter of diffuse large B-cell lymphomas (DLBCL) affects individuals of 75 years of age or older. Nevertheless, data on optimal treatment of these patients is scarce, mainly because very elderly patients are usually excluded from clinical trials due to poor performance status and co-existing medical conditions. According to previous studies, replacement of adriamycin with epirubicin in CHOP regimen has proven equally effective in the treatment of aggressive non-Hodgkin lymphomas (NHL), yielding at the same time lower rates of cardiac and hematological toxicity. Rituximab, on the other hand, is well-known for its effectiveness and good tolerance in the treatment of elderly patients with aggressive NHL. Aim: To study retrospectively the efficacy and safety of CEOP regimen ± rituximab in very elderly patients with DLBCL. Methods. Between 1998 and 2005, 45 patients, 20 (44.4%) males and 25 (55.6%) females with median age of 78 (75-85) years were diagnosed with DLBCL in our department. Twenty-one (46.7%) of them had DLBCL of nodal origin and 24 (53.3%) of primary extranodal origin. Twenty-seven (60%) patients presented with early stage (I-II, no X) disease and 55 (75.3%) with IPI 1-2. Nine (20%) patients had B symptoms, 5 (6.7%) bulky disease, 2 (4.4%) bone marrow infiltration and 12 (26.7%) extra-nodal involvement other than primary. All patients received cyclophosphamide 750 mg/m² IV, epirubicin 62.5 mg/m² IV and vincristine 1.4 mg/m² IV on day 1 and prednisone 75 mg IV on days 1-5. Doses were reduced (mildly) due to age > 80 years medical history of heart disease, by 25% in 15 (33.3%) and by 50% in 5 (6.7%) patients. Rituximab was administered on day 1 of each chemotherapy cycle at a dose of 375 mg/m² IV, in 23 (51.1%) patients. Fifteen (33.3%) patients also underwent radiation therapy. All patients were under close haematological and cardiac monitoring throughout treatment. Disease-free survival (DFS), overall survival (OS) and failure-free survival (FFS) were estimated according to the Kaplan-Meier method. Results. Median follow-up time was 30 (1-105) months and median number of chemotherapy cycles administered was 6 (1-8). On an intention-to-treat basis complete response was observed in 33 (73.3%) patients, partial response in 5 (11.1%), stable disease in 2 (4.4%) and progressive disease in 1 (2.2%), whereas 3 (6.7%) patients were not evaluable. Sixteen (35.6%) patients are dead and 27 (60%) are alive, 17 (63%) of which, in remission. Actuarial DFS, OS and FFS rates at 3-years were 75.8%, 67.3% and 53.8% respectively. OS and FFS rates at 5-years were significantly (p < 0.003) higher in responders (76.7% and 60.3% respectively) than non-responders (19.3% and 22.9% respectively). No treatment-related deaths were noted, while hematological and cardiac toxicity remained acceptable. Conclusion: CEOP ± rituximab is a feasible, safe and effective treatment for very elderly patients with DLBCL. The high response and survival rates in our study justify the right of these patients to a potentially curative treatment.

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EFFICACY OF EPOCH AND RITUXIMAB-EPOCH AS SALVAGE THERAPY FOR RELAPSED OR REFRACTORY B-CELL LYMPHOMA: PRELIMINARY RESULTS OF AN OPENED, NON-RANDOMIZED STUDY IN A SINGLE CENTER
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Backgrounds. Relapsed or refractory B-cell Non-Hodgkin lymphoma (NHL) have a poor prognosis. EPOCH chemotherapy and rituximab-EPOCH chemotherapy have been used in advanced stage or relapsed/refractory NHL, but the response rate and survival of rituximab have not been adequately defined in such cases. Aims. To preliminarily evaluate efficacy of EPOCH and R-EPOCH regimens in terms of response rate, survival and toxicities. Possible predictive factors for response were also assessed. Methods. Thirty-six patients with relapsed or refractory B-cell NHL were enrolled in Songklanagarind Hospital during January 2003 and January 2006. All of patients received conventional CHOP chemotherapy without rituximab as a first-line treatment. In an opened, non-randomized way, 25 patients received EPOCH (doxorubicin 10 mg/m², etoposide 50 mg/m² vincristine 0.5 mg as a continuous IV infusion on days1-4, cyclophosphamide 750 mg/m² IV on day 5 and prednisone 60 mg/m² orally on days 1-5) and 11 patients received R-EPOCH (addition of rituximab 375 mg/m² intravenously 24 hr before EPOCH regimen). Results. The patient characteristics were not statistically different between EPOCH group and R-EPOCH group, in term of age, gender, histopathology, stage of disease, IPI, B-symptoms, performance status, bulky mass, elevated LDH, prior response to the first-line chemotherapy, and duration from diagnosis to salvage treatment. Of 23 evaluable patients with EPOCH treatment and of 11 patients with R-EPOCH, objective responses were obtained in 52% (35% CR, 17% PR) and 75% (64% CR, 9% PR), respectively (p=0.30). There were no significant predictive factors of response as a function of histopathology, stage of disease, IPI, B-symptoms, performance status, bulky mass, elevated LDH, prior response to the chemotherapy history, high-dose chemotherapy infusion-related reactions occurred in 2 patients (18%). Febrile neutropenia developed in 12 of 216 cycles (6%). Cardiotoxicity was about 8%. Because of short duration of follow-up (median, 7.8 mo for EPOCH and 10.6 months for R-EPOCH), EFS and OS could not be appropriately analysed at this report. Conclusion: EPOCH and R-EPOCH regimens were effective and well tolerated in patients with B-cell NHL who were relapsed from or resistant to the conventional chemotherapy. R-EPOCH seemed to give higher response rate that EPOCH but it is not statistically significant. Because of this preliminary result, more number of patients and longer period of follow-up are needed.

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IN VIVO AND IN VITRO PURGING WITH RITUXIMAB PLUS CHEMOTHERAPY, CD34+/CD133- CELL SELECTION AND HIGH DOSE CHEMOTHERAPY AS CONSOLIDATION TREATMENT IN ADVANCED MANTLE CELL LYMPHOMA
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Backgrounds. Mantle-cell lymphoma (MCL) remains an incurable lymphoproliferative disorder with standard chemotherapy (CT). The chimeric monoclonal antibody anti-CD20 Rituximab has demonstrated to improve the CR rate representing an interesting in vivo purging modality; however most pts treated with standard CT relapse within 2 years and the role of consolidation therapy is crucial. In vitro purging might contribute in obtaining a molecular (bcl-1) CR which could be predictive
for improved outcome. Aims. Here we considered the efficacy of an intensified in vitro and in vitro purging consolidation program based on Rituximab with CT, CD34+ positive selection after PBSCT collection, followed by HD CT in advanced and refractory MCL pts. Methods. From October 1999 to December 2003 we treated 13 pts (9 newly-diagnosed, 4 previously-treated) with a median age of 55 yrs (range 41-66). At the time of diagnosis 11/13 had IV stage, 5 with extranodal involvement and in 7/13 the molecular biological analysis showed a high level of positivity in 11 pts. Pts received Rituximab (375 mg/m²) at the first day of each of two ChOP-like cycles followed by Cyclophosphamide 4 g/m² and G-CSF to collect >2 x 10^6 CD34+ cells/kg (apheresis were processed by CliniMACS for CD34+/CD133+ cell purification) and then by 2 BEAM and CD34+ cell reinfusion after ida 15 mg/m² and Melphalan 180 mg/m². Results. All patients completed the scheduled treatment without any major toxicity and were fully evaluable for clinical response. Before HD CT, 4 pts achieved CR and 9 were in PR. After transplantation 12 pts were in CR and 1 in PR. With a median follow-up of 45 months (range 27-70), 7 pts (54%) are still in CR (confirmed by PCR). Considering the in vivo/in vitro purging effect, among the 11 bcl-1 positive pts at baseline, 6 were still bcl-1 positive before stem cell collection; we did not perform in vitro purging in 2 pts because of low number of CD34+ collected; 2 out of 4 contaminated apheresis became bcl-1 negative after in vitro purging. After transplant, 4/7 of pts who received bcl-1 negative apheresis maintain a molecular CR, versus 4 who received bcl-1 positive apheresis. Conclusions. Our data seem suggest that an intensive chemo-immuno consolidation therapy can improve the outcome in advanced MCL pts. Five Rituximab courses as conventional schedule do not appear sufficient to induce a molecular CR; on the other hand the reinfusion of bcl-1 positive cells does not hinder the possibility to achieve a CR and conversely purging in vitro can actively influence the engraftment especially for platelet recovery. The use of different schedules of Rituximab, its employment in the conditioning regimen and as maintenance therapy, could represent a new strategy for pts belonging to this unfavourable risk group.

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**RECOMBINANT URATE OXIDASE (RASPURICASE) FOR THE PREVENTION AND TREATMENT OF HYPERURICEMIA DURING CHEMOTHERAPY FOR HEMATOLOGICAL MALIGNANCIES**

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Tumor lysis syndrome (TLS) is a serious complication of the induction therapy of lymphomas and leukemias. The standard approach for the prevention and management of hyperuricemia is hydration, oral allopurinol and alakalinaization. Urate oxidase is an enzyme that catalyses the conversion of uric acid (UA) to allantoin which is 5-10 times more soluble than UA and therefore is excreted by the kidneys more easily. It is found in most mammals but not in humans. Rasipurase, a recombinant form of urate oxidase catalyses the conversion of uric acid to allantoin and therefore controls hyperuricemia faster and more efficiently than allopurinol. To evaluate retrospectively the safety and efficacy of rasipurase in patients with hematological malignancies who were treated with chemotherapy and had increased risk to develop tumor lysis syndrome. We studied 21 patients (16 men and 5 women) with median age 65 years (range 26-76). They had Burkitt lymphoma (n=5), lymphoblastic lymphoma (n=2), mantle cell lymphoma (n=1), diffuse large B-cell lymphoma (n=5), Hodgkin lymphoma (n=2), chronic lymphocytic leukemia (n=4), acute leukemia (n=4) and received rasipurase during the first therapy for their disease. Before treatment 76% of the patients had increased lactate dehydrogenase (LDH), and 52% increased uric acid. Rasipurase was administrated intravenously at a dose of 0.20 mg/kg/day for 2 to 7 days, starting from the first day of each chemotherapy. The results of this study confirm the efficacy and safety of rasipurase for the prevention and treatment of hyperuricemia induced by chemotherapy in patients with hematological malignancies.
The principal aim of this study is to present the epidemiological data, clinical characteristics, clinical and histological staging, response to chemotherapy and define the role of irradiation and the final outcome. Patients and results: During the last 8 years 24 children, 17 boys and 7 girls, aged 4.5 to 15 years old were diagnosed with Hodgkin Lymphoma in the Department of Pediatric Hematology - Oncology of the ‘Aghia Sofia’ Children’s Hospital. Thirteen out of twenty-four (54%) patients had B-symptoms, while 16/24 have been admitted with signs of inflammation of cervical and supraventricular lymph nodes. The 3 out of 8 patients with B-symptoms did not present peripheral lymphadenopathy. In all our patients the diagnosis was established with biopsy material. In all patients bone marrow biopsy was performed. Of note, one of them was diagnosed by bone marrow involvement. Twenty out of twenty-four (10/24) and 19/24 underwent Galium lymph node and Technetium bone Scann, respectively. Regarding pathology type, 17/24 patients had nodular sclerosing type, six (6) mixed cellularity and one (1) lymphocyte predominant histology. Thirteen out of twenty-four patients had positive Galium Scann and while three (3/24) were positive for bone involvement by Technetium Scann. Clinical staging: 3 children were staged as stage I (3/3 IA), 10 stage II (3/10 IIB), 6 stage III (4/6 IIIA) and 5 stage IV. Igeneen out of twenty-four (18/24) of the patients were treated according to the German protocol GPOH-HD-95, while 5 were treated with cycles of the MOPP/ABV regimen. Radiotherapy was administered to the involved sites in 24/24 patients (with bulky disease 3/12, residual masses 3/12, advanced stage 2/12 and local relapse 4/12). Four out of twenty-four (5/24) experienced relapse (3 stage IV all with mixed cellularity histology and two patients stage III with nodular histology). All relapsed patients were treated with chemotherapy followed by autologous bone marrow transplantation. Overall 22/24 children survived today (17 CR1, 2 CR2, 1 CR3 and 2 SD) with a median follow-up 54 months (range 4 to 120 months). One patient is lost follow-up and 1 is dead 24 months after the diagnosis. Conclusion: We report the survival of our patients with Hodgkin Lymphomas after their first or second remission is standing high. More patients are salvage following relapse. The longterm follow-up of children with HD concerning possible complications of therapy remains a serious issue. We believe this study offers useful clinical information about staging, appropriate treating decision and outcome of the disease in the longterm inclusion.
leukalidomide in the lenalidomide-sensitive chromosome 5 deleted Burkitt’s Lymphoma tumor cell line, Namalwa CSN.70, with various chemotherapeutic agents used for oncological treatment (cyclophosphamide, doxorubicin, vincristine, methotrexate, cytarabine, ifosfamide, carbustine, prednissone and etoposide). Methods. Cells were incubated in 96-well culture plates with compounds for 72 hours and assayed by SH-thymidine incorporation. IC50s were calculated by nonlinear regression in GraphPad Prism. Results. Namalwa cell proliferation was inhibited by the chemotherapeutic agents doxorubicin, vincristine, methotrexate, cytarabine, carbustine, prednissone and etoposide. Ifosfamide and cyclophosphamide had no anti-proliferative effects on Namalwa cells. However, lenalidomide in combination with these agents produced varied responses on Namalwa cell proliferation. Specifically, lenalidomide in combination with cytarabine, doxorubicin, or vincristine generated anti-proliferative responses that were equivalent to the inhibition produced by these respective chemotherapeutic agents alone. Lenalidomide in combination with cyclophosphamide or ifosfamide produced anti-proliferative effects similar to lenalidomide alone. These data indicate non-additive effects for the previously mentioned lenalidomide/chemotherapeutic agent combination. However, the lenalidomide/etoposide combination yielded partially additive anti-proliferative effects within the concentration range of 0.05 and 0.5 mM. At higher concentrations, > 0.5 mM, the response became non-additive and comparable to the etoposide treatment alone. Also, the lenalidomide/prednissone combination resulted in partially additive anti-proliferative effects but at concentrations > 0.5 mM and was non-additive at lower concentrations. Antagonistic effects were observed with the lenalidomide/carmustine combination at low concentrations while partially additive anti-proliferative effects were observed at higher concentrations. Antagonistic effects were also observed with the lenalidomide/methotrexate combination. Conclusions. Together, these results suggest there was trend of divergence in Kaplan-Meier’s survival curves after a four years follow-up (log rank p<0.05). Conclusions. The patients with high Ki67+ are at risk of relapse and treatment failure, and are eligible for the initial aggressive therapeutic approach.

1392 INCIDENCE AND CLINICAL SIGNIFICANCE OF AUTOIMMUNE COMPLICATIONS IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA AND NON-HODGKIN’S LYMPHOMA

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Background. Autoimmune complications (AC) as autoimmune hemolytic anemia (AIHA) and immune thrombocytopenic purpura (ITP) can be present in evolution of chronic lymphoproliferative disorders (CLPD) or they, sometimes, precede the diagnosis. The outcome of patients with CLPD associated with AIHA or ITP has been reported, in previous studies, to be similar to the outcome of patients without AC. The aim of our study was to evaluate the incidence and clinical significance of AC in patients with B-cell chronic lymphocytic leukemia (CLL) and in patients with non-Hodgkin’s lymphoma (NHL). Methods. In a retrospective analysis we studied 384 patients with CLL (165 pts) or NHL (219 pts) diagnosed and treated in a single institution between 1990-2005. Clinical and hematological data were reviewed for patients with AC. Results of incidence, characteristics, clinical response, disease stage, histological type, and treatment. Results. AC were found in 31 of 384 patients (8.07%), in 17 (10.80%) of 165 CLL patients and in 14 (6.39%) of 219 NHL patients. AIHA was present in 18 patients (58.06%), 11 in 7 patients (22.5%), while in 6 patients (19.85%) an ITP was observed. The most common AC (AIHA associated with ITP) was diagnosed. AC were more frequent in CLL than in NHL: AIHA: 70.58% vs 42.85%; ITP: 29.41% vs 14.28%. In 3 cases (9.67%) 2 CLL and 1 NHL AIHA was diagnosed before the diagnosis of CLPD. Most of AC appeared in the advanced stages of the diseases (76.47% CLL stage C; 64.28% NHL stages III-IV). The median lymphocyte count at diagnosis, in the 17 patients with CLL, was 48,9×109/L (7.8 – 188.1). Of the 14 patients with NHL, AC were diagnosed in 3 cases (21.42%) with small lymphocytic lymphoma, in 2 cases (14.28%) with follicular lymphoma, and in 9 cases (64.28%) with diffuse large B-cell lymphoma. The successful treatment of AC included corticosteroids (96.77%) and/or chemotherapy (19.38%). The outcome of patients with AC was not different from the outcome of patients without AC. Nine (52.94%) of the 17 CLL patients, and 8 (57.14%) of the 14 NHL patients reached a complete response after adequate chemotherapy. The median survival rates were 4.8 years for CLL patients and 3.5 years for NHL patients. No death related to AC was recorded. Conclusions. B-cell CLL and NHL are associated with AC, and the incidence of this AC is low. Although most of AC are diagnosed concurrently especially in advanced stages of the CLPD, sometimes they can precede the diagnosis of CLL or NHL. The majority of patients responded well to corticotherapy and/or chemotherapy. The outcome (therapeutic response, survival) and prognosis are similar to other patients with CLL or NHL.

1393 DIFFERENT PROFILES OF ADHESION MOLECULES IN B-CELL NON-HODGKIN’S LYMPHOMA (B-NHL) ARE ASSOCIATED WITH PERIPHERAL BLOOD INVASION


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The frequency of the leukemic phase is different in the various histological subtypes of B-NHL. In chronic lymphocytic leukemia (CLL) the involvement of peripheral blood is always present; in mantle-cell lymphoma (MCL), the leukemic phase is present in 50%-70%; and in nodal and splenic marginal B-cell lymphoma (MZL) in about 10% and 50%, respectively. We hypothesized that the down-regulation of some adhesion markers could contribute to the leukemic dissemination observed in some B-NHL subtypes. We evaluated the expression of 10 adhesion molecules in tumor cells of peripheral blood of 17 patients with CLL, 17 with MCL, and 13 with nodal or splenic MZL, all in leukemic phase. All cases of CLL had 4 or 5 points in the Matutes scoring system, while MCL and MZL cases had between 0 and 3 points. In addition, all MCL cases had evidence of CYCLIN D1 overexpression. The mean fluorescence intensity (M.I) of the adhesion molecules in tumor cells was measured by flow cytometry in CD19-positive cells. The M.I of CD11a, CD11b, CD11c, CD18, CD49c, CD49d, CD29 and CD54 were different in the three groups (Table).
The Dunns post test was applied when the p value was <0.05. The comparison between CLL and MCL showed that CLL presented a higher expression of CD11c and CD49c, and a lower expression of CD11b and CD49d. When we compared the CLL with MZL, the CLL showed a higher expression of CD49c and lower expression of CD11a, CD11b, CD18, CD49d, CD29 and CD54. Finally, the comparison between MCL and MZL showed that the MZL had a higher expression of CD11a, CD11c, CD18, CD29 and CD54. The structure of normal lymphoid follicle in lymph nodes seems an appropriate association between the B-lymphocytes and the dendritic follicular cell through the interaction between CD11a and ICAM-1, as well as CD49d and VCAM-1. The lower expression of CD11a and CD49d on CLL cells could facilitate their detachment from the lymph node to invade the peripheral blood. A higher frequency of splenic involvement has been reported in cases of CLL with strong positivity to CD11c. However, in our series 82% of the MCL patients presented with an enlarged spleen, but showed the lowest expression of CD11c among the groups. Thus, our findings give support to the role of adhesion molecules in the determination of nodal or leukemic presentation in lymphoid malignancies.

**Table:** Median values of M.F.I. of adhesion molecules.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>CLL</th>
<th>MCL</th>
<th>MZL</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD11a</td>
<td>167.8</td>
<td>251.7</td>
<td>401.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CD11b</td>
<td>0</td>
<td>37.7</td>
<td>51.7</td>
<td>0.05</td>
</tr>
<tr>
<td>CD18</td>
<td>158.0</td>
<td>356.0</td>
<td>518.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CD49a</td>
<td>97.7</td>
<td>148.2</td>
<td>198.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CD49d</td>
<td>81.7</td>
<td>118.2</td>
<td>138.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CD54</td>
<td>314.2</td>
<td>331.2</td>
<td>341.2</td>
<td>0.05</td>
</tr>
<tr>
<td>CD29</td>
<td>232.4</td>
<td>218.6</td>
<td>228.6</td>
<td>0.05</td>
</tr>
<tr>
<td>CD11c</td>
<td>4.2</td>
<td>1.2</td>
<td>0.2</td>
<td>0.05</td>
</tr>
</tbody>
</table>

**1395**  
**RISK OF CANCER IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA. EXPERIENCE AND REPORT FROM A RETROSPECTIVE STUDY IN HEMATO-ONCOLOGY DEPARTMENT, UNIVERSITY HOSPITAL OLOMOUC**

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**Background:** B-cell chronic lymphocytic leukemia (B-CLL) is characterized as a chronic indolent disease with an immunodeficiency. It is the most common leukemia of adult people, especially in the elderly. It is known that immunodeficiency and age can be the risk factors of cancer. Based on these facts we analyzed retrospectively our own data from patients with B-CLL who were diagnosed in our centre from 1994 to 2004.

**Patients and Methods:** We analyzed group consisting of 215 patients, male/female 129/86. The median age of patients was 64 (35 - 91), in the clinical stage Binet A 123 (57%) patients, Binet B 55 (26%) patients, Binet C 37 (17%) patients. There were 19 patients who had a malignancy before the diagnosis of B-CLL was determined such as melanoma (4), Grawitz's tumor (3), colorectal cancer (2), basal cell carcinoma (2) and squamous cell carcinoma (1), uterous carcinoma (1), cancer of breast (1), lung (1), stomach (1), prostate (1), parotid gland (1), osteochondroma (1), leiomyosarcoma (1) and urinary bladder papiloma (1). Two patients had two of these tumors as listed above. There were 15 patients who developed second solid tumors after a diagnosis of B-CLL was established and 4 of them did not receive any chemotherapy for B-CLL. These second tumors involved basal cell carcinoma (5), colorectal carcinoma (2), cancer of the thyroid gland (2), lung (2), kidney (2), prostate (1), squamous cell carcinoma (1), uterous carcinoma (1) and bone metastasis (1). The incidence of all solid cancer was in 34 patients (16%), ratio male/female - 20/14 with a median age of 69. Most of these solid tumors were diagnosed in the clinical stage Binet A in 18 patients (53%), Binet B in 8 patients (24%), Binet C in 8 patients (24%). Conclusion. The development of second solid cancers in B-CLL diagnosed patients represents a high risk factor and a complication among long term survivors. Longer follow-up is needed to assess proper anamnesis, physical examination (skin lesion included) and diferential diagnosis.

**1396**  
**MULTIPLE MYELOMA IN A PATIENT WITH CHRONIC LYMPHOCYTIC LEUKEMIA. EVIDENCE OF A COMMON PATHOLOGICAL CLONE**

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The coexistence of chronic lymphocytic leukemia (CLL) and Multiple Myeloma (MM) in the same patient is very rare. It is uncertain whether the myeloma cell represents a clonal evolution of the CLL cell or a totally different cell population. We present one female, 62 years old patient suffering from CLL. From 1998 to 2005 she has received therapy for the CLL (chlorambucil and fludarabine in combination with cyclophosphamide). On March 2005 she was presented with pancytopenia and the diagnosis of multiple myeloma was established. In the bone marrow aspirate a diffused infiltration from 40% λ- monoclonal myeloma cells and 40% interstitial infiltration from CLL cells was found. The immunophenotype showed the same light chain in both the MM and CLL bone marrow cells examined. The G-banding conventional karyotype and the molecular cytogenetic analysis by M-FISH and M-BAND showed two different clones. One clone with 45 chromosomes and t(13;14)(p11;p11) and an other with the same chromosomal abnormality and additional complex chromosome rearrangements such as deletion of one chromosome 4, t(4;9), t(4;9;15), t(6;9;15), t(6;11), t(8;17), and t(16;21). The patient underwent chemotherapy with thalidomide plus dexamethasone and had a short partial remission of both diseases. Finally, she died on August 2005. Conclusion. The fact that the neoplastic cells carried the same light chain and the presence of translocation

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1937 IMMUNE THERAPY IN CHRONIC LYMPHOCYTIC LEUKEMIA: ALEMTUZUMAB (MABCAMPATH) (ANTI-CD52)

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Backgrounds. Chronic lymphocytic leukemia (CLL) is the most prevalent leukemia in adults in The Netherlands. Recent developments with monoclonal antibodies offer new therapeutic options. Alemtuzumab (MabCampath™) is a monoclonal antibody aimed at CD52, which is present on both normal and abnormal B and T lymphocytes. It is indicated as third line therapy in the treatment of CLL, after failure of conventional therapy including fludarabine. The most important side effects of Alemtuzumab treatment are opportunistic infections (pneumocystis carinii pneumonia (PCP) and CMV pneumonitis). It is unclear whether these complications do indeed lead to problems in the treatment of CLL patients in the Netherlands. Aims. To gain insight in the use and complications of Alemtuzumab in the Netherlands. Methods. A questionnaire concerning the treatment of CLL patients with Alemtuzumab was made on the basis of the literature [1] and sent to 11 hospitals in The Netherlands. Results. From 18-02-02 until 01-04-05 22 patients (mean age 64 years, 16 men, 6 women) with CLL RAI/BINET stage IIA to IV were treated with 26 treatments of Alemtuzumab according to schedule (starting dosages 3, 10, 30 mg, followed by 3 times per week 30 mg i.v./s.c. for 4-12 weeks). Patients had received a mean of 3 lines of previous therapy before starting on Alemtuzumab. The time from diagnosis until the start of Alemtuzumab treatment was 5.6 years (4.5) (mean, SD). The duration of treatment was 9 (5.4) weeks (mean, SD). Reasons for early discontinuation of therapy were: fever and other side effects 20%, progressive disease (PD) 13%, complete response (CR) 13%, bone marrow toxicity 13%, other reasons 7%, unknown 38%. 27% of the treatments could be continued for the full 12 weeks. The most prevalent side effects were fever 78%, rigor 49%, dyspnea 19% and tiredness 15%. Infectious complications were pneumonia 26.9% (of which 1 PCP), sepsis 7.7%, herpes zoster 7.7%, sinusitis 7.7%, meningitis 3.8%, guillain barre 3.8%, others 15.3%. The response attained was CR 17%, partial response 35%, stable disease 30% and PD 17%. The duration of the response was 9.5 (7) months (mean, SD). Summary/Conclusions. Treatment with Alemtuzumab is often discontinued prematurely. Therefore the maximal therapeutic effect cannot be reached. Fear of severe uncontrollable opportunistic infections seems unfounded.

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References

1939 ANALYSIS OF RISK FACTORS OF 248 PATIENTS WITH B-CHRONIC LYMPHOCYTIC LEUKEMIA AT DIAGNOSIS

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248 patients (159 men and 109 women) with B-cell chronic lymphocytic leukemia were evaluated at diagnosis with respect of clinical stage, CD38 and ZAP-70 expression, cytogenetic changes (by FISH method) and IgVH mutation status and impact of these for overall survival. In Rai 0 and I clinical stage were 203 patients (82.9%), in stage II were 25 patients (10.2%) and in stage III and IV were 17 patients (7.0%). CD38 expression was evaluated at 137 patients (55.2%), positive was for 49 patients (55.8%), negative was at 88 (64.2%) patients. ZAP-70 expression was evaluated at 109 patients (44% from the total number), positive was at 44 patients (40.4%), negative at 65 (59.6%) patients. From 160 evaluations of IgVH mutation status (64.5% of the total number of patients) 71 (44.4%) patients were non-mutated and 89 (55.6%) cases were mutated. From 192 evaluated patients (77.4% of the total number of patients) trisomy of 12 chromosome was present at 22 patients (11.5%), one case was borderline (0.5%), 13(q14) deletion was present at 107 cases (56.6%) out of 189 evaluated (76.2% of the total number of patients), 11(q23) deletion was found at 4 cases (12.9%) out of 31 evaluated (12.5% of the total number of patients). 13(q23) deletion was present at 13 cases (16.5%) out of 79 evaluated (31.9%) and 17(p13) deletion by 22 patients (11.4) out of 193 evaluated (77.8%). 48 patients died (19.4%), overall survival in 5 years was 83.5%, in 10 years 58.2%. According to our analysis sex (p=0.0002), Rai clinical stage (p=0.0002), IgVH mutation status (p=0.0001), 17(p13) deletion (p=0.09), CD38 (p=0.05) and ZAP-70 expression (p=0.02) revealed to be significant prognostic risk factors.

1939 HALF OF CLL PATIENTS REQUIRING THERAPY DISCLOSE NORMAL FISH KARYOTYPE OR THE FAVORABLE 13q DELETION

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Chromosomal aberrations, detected by FISH, are considered as one of the most important prognostic factor in B CLL. Due to its expensive cost we chose to focus our analysis only on patients who required therapy. Fifty-five patients were studied, including 12 patients in stage Binet A and showing progressive disease. The lymphocytes were fixated for analysis before starting cytoreductive therapy. Examination included: 13q, 17p and 11q deletions and chromosome 12 trisomy. The results are presented in figure. The 17p deletions were found in 5 cases, including 2 active PLL patients and 3 CLL patients in stage Binet C. Trisomy 12 was found in 9 patients, all except one exhibited CLL/PL or PLL morphology. The 11q deletions were found in 5 Binet C patients. The 13q deletion was found in 13 patients including 4 in stage Binet A, 5 in stage B, 3 in stage C and 1 with aggressive PLL.

In addition, 13q deletion was associated with 17p del (2 patients), 11q del (4 patients) and 12 trisomy (8 patients). Single case disclosed 12+;11q del. Overall, chromosomal aberrations other than 13 del, were found in 4 out of 12 patients diagnosed in stage Binet A. FISH didn’t show any aberrations in 15 cases. Considering the high prognostic significance of FISH analysis in CLL requiring therapy, it would be expected that patients in advanced stages and with progressive disease have unfavorable results. Nevertheless, our analysis in CLL patients requiring therapy showed that FISH results do not always correlate with the clinical stage of the disease. Part of the patients in stage Binet A had chromosomal aberrations supporting the need for therapy, but in other cases FISH revealed a favorable profile. Altogether, half of our patients disclosed either normal FISH results or the favorable 13q del. In conclusion, decision for treatment in patients with CLL cannot rely on FISH analysis alone and should be accompanied by additional prognostic factors.
1400

**VH GENE USAGE AND SOMATIC MUTATION DISTRIBUTION CONSISTENT WITH ANTIGEN-DRIVEN SELECTION IN BOTH ‘MUTATED’ AND ‘UNMUTATED’ CASES OF B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA**

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In B-cell chronic lymphatic leukaemia (CLL), part of the cases shows mutated VH genes indicating that the transformed B-cell has passed through the germinal centre where it has undergone the somatic hypermutation machinery during an antigen (Ag) response. In order to examine the possible role of Ag stimulation in the pathogenesis of B-CLL, we analysed 40 VH sequences derived from 37 CLL patients. VH genes were amplified, sequenced and aligned with all known germline VH genes available on the internet (IgBlast and IMGT). The observed number of replacement (R) mutations within the complementarity-determining regions (CDRs) and the framework regions (FRs) (ObsR CDR and ObsR FR) were compared with the calculated expected numbers (ExpR CDR and ExpR FR), taking into account the inherent replacement susceptibility of CDRs and FRs. The probability (p value) that scarcity or excess of R mutations resulted by chance only was calculated for CDRs and FRs using the binomial distribution model. VH gene usage was biased with exclusive use of VH3 (20), VH4 (11) and VH1 (9) genes. VH4-34 and VH3-30 genes were respectively 6 and 4 times used. For 23 of 26 mutated VH genes (homology < 98%), either the ObsR CDR was higher than the ExpR CDR or the ObsR FR was lower than the ExpR FR with p values < 0.05 in 10 cases, indicating evidence for positive and/or negative selection. The non-amplified VH sequence showed evidence for Ag selection. The preferential usage of certain VH genes as well as the skewed distribution of R mutations indicates that certain Ags may be involved in the pathogenesis of CLL. The VH4-34 gene, most frequently used in this series, encodes for an auto-reactive immunoglobulin (Ig) that is associated with lymphocyte malignancies, particularly EBV. Further studies of the binding sites of restricted Ig, are necessary to elucidate the possibility of Ag involvement in CLL development. Furthermore, as evidence of antigen selection is detected in both mutated and unmutilated CLL cases, its role in predicting prognosis, in addition to the VH-mutation status, should be investigated.

1401

**FLUDARABINE-IFOFSAMIDE-RITUXIMAB (FIR): A THERAPEUTIC OPTION FOR CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS RESISTANT TO CHLORAMBUCIL**

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Ifofsamide is an alkylator belonging to the oxazophosphorins whose efficacy, alone or in combination as mobilizing agent or as salvage therapy, has been demonstrated in solid tumours and lymphoma but not yet in CLL. Fludarabine is effective in CLL patients refractory/relapsing after chlorambucil and its effectiveness in this disease is enhanced by the association with cyclophosphamide (FC) and monoclonal antibodies, in particular Rituximab (FCR combination). To evaluate the efficacy and safety of a Fludarabine Ifosfamide and Rituximab-containing regimen in pretreated CLL patients, showing a sustained and durable response rate with an acceptable incidence of adverse events, suggest that a chemotherapeutic regimen combining Fludarabine, Ifofsamide and Rituximab may be a feasible therapeutic alternative in patients affected by relapsing and refractory CLL.

1402

**HAIRY CELL LEUKAEMIA: RETROSPECTIVE CLINICAL STUDY.**


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Background and objectives: The hairy-cell leukaemia (HCL) is an infrequent disease with indolent course generally benign and with a good therapeutic response using purine analogues. We have evaluated the presentation form and clinical evolution of this disease. Methods: We reviewed 17 hairy cell leukaemia cases were diagnosed between 1983 to 2005 by flow cytometry and by electron microscope. We revised the clinical evolution and therapeutic response. Results: We reported 17 HCL cases, 3 variant form. Mean age of presentation was 60.2 years (28-35). Sex was predominantly male (62.4%) and the more frequent cause of diagnosis was any analgesic cytopenia: moderate thrombocytopenia (<120.000 platelets/mm³) (12 cases), with a mean of 89.000 platelets/mm³. Leucopenia (<1500 leucocytes/mm³), was present in 3 cases, granulocytopenia was more frequent (median of 70% with 70% <1000 granulocytes/mm³). Anemia (< 12 g/dl of hemoglobin) 8 cases. Splenomegaly at diagnosis appeared in 64.3% of the cases. Asthenia or repeated infections appeared in 58.8% of the patients. The median of HCL cells in blood was 24.9% (1-61%) at diagnosis (15 by flow cytometry and 2 by electron microscope). In relation to treatment and evolution, 2 patients with stable disease did not receive treatment, 10 cases were treated with cladribine, 3 with interferon, 1 with chlorambucil, and 1 with splenectomy. The global response was 94.1% (41% CR, 41% PR and 12% Stable Disease). The median response time was 15 months. The frequency of relapse was high (47.6%) with interferon more frequent with interferon (100%) than with cladribine (53%). However the response to rescue therapy was good (87.5%; 62% with cladribine and 33% with interferon). Three patients died but only two in relation with the disease (infectious disease). The median free event survival is 40 months and the global survival 168 months. Conclusions. HCL is an indolent usually diagnosed by analytical methods. The treatment response is high, but with frequent relapses. However the response to rescue is excellent. HCL has a long survival though the age of presentation is advanced.

1403

**IMPACT OF TRISOMY 12, DEL(13Q), DEL(17P) AND DEL(11Q) ON THE IMMUNOPHENOTYPE, DNA PLOIDY STATUS AND PROLIFERATIVE RATE OF LEUKEMIC B-CELLS IN CHRONIC LYMPHOCYTIC LEUKEMIA**


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B-cell chronic lymphocytic leukaemia (B-CLL) is a well-defined clinical entity which displays a variable clinical course in association with the existence of heterogeneous molecular and cytogenetic features. To analyze the relationship between the presence of trisomy 12, 13q-, 17p- and 11q- and the immunophenotype, DNA ploidy status and proliferative...
tive characteristics of neoplastic B-CLL cells. The impact of trisomy 12, del(13q), del(17p) and del(11q) was determined by interphase fluorescence in situ hybridization analysis (ISH) of purified neoplastic B-cells from a series of 180 patients with newly diagnosed B-CLL on the immunophenotype, DNA ploidy status and the proliferative rate of neoplastic B-cells. Half (50%) of all B-CLL cases studied displayed one (40%) or more (10%) of the genetic abnormalities, trisomy 12 and del(17p) being the most frequently detected ones (25% and 21%, respectively), del(17p) and del(11q) being found in 9% and 9.4% of the cases, respectively. Trisomy 12 was associated with a higher frequency of DNA aneuploidy (p=0.012) together with a higher reactivity for CD22, CD27, CD24 and CD79b. The expression of the last latter marker was also higher among cases with 17p- which in turn showed reduced CD11c. Cases carrying del(13q) showed a high expression of VEGF of CD5, CD43 and CyBCL2, these latter two markers being also brighter among cases with 11q-. Remarkably, none of the chromosomal abnormalities investigated was associated with an increased proliferation of neoplastic B-cell by itself, although B-CLL cases simultaneously showing 13q- and 17p- displayed a higher percentage of S+G2/M-phase tumor cells as compared with individuals carrying either 13q- or 17p-. The latter two markers being also brighter among cases with 11q-. In summary, our results confirm and extend previous observations about the frequency of trisomy 12, 13q-, 17p- and 11q- in B-CLL patients, where they affect only a variable proportion of all neoplastic cells showing that the abnormalities found in B-CLL patients do not impact on the immunophenotype profile of B-CLL cells; in contrast, the impact of these cytogenetic abnormalities on the proliferative rate of neoplastic B-cells was only noted for cases simultaneously carrying 13q- and 17p-.

1404 ANGIogenic cytokines in B-CELL CHRONIC LYMPHOCYTIC LeUKEMIA: ASSOCIATION WITH IGHV MUTATION STATUS AND GENETIC ABNORMALITIES

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Backgrounds. B-cell chronic lymphocytic leukemia (B-CLL) is a disease with an extremely variable clinical course. New prognostic factors such as mutation status of immunoglobulin heavy chain variable region (IGHV) or genetic aberrations detected by fluorescent in situ hybridization (FISH) are being increasingly used in order to identify patients with high-risk disease. Several studies have shown that anogenesis is increased in B-CLL and may potentially help in prognostic assessment of B-CLL patients. AIM: To assess relationship between plasma concentrations of vascular endothelial growth factor (VEGF) or basic fibroblast growth factor (bFGF) and IGHV mutational status or genetic aberrations. Methods. We measured VEGF and bFGF using sandwich enzyme-linked immunosorbent assay (ELISA) kits in peripheral blood plasma of 49 patients (males, females, age) with untreated B-CLL and 50 healthy donors. IGHV mutation status was analyzed using cDNA transcribed from B-CLL RNA for touch-down reverse transcription polymerase chain reaction (RT-PCR) with degenerate primers for VH1-7 families; IGK sequences were aligned to the nearest gumble using the Ig BLAST database. There were 26 patients with low risk, 17 with intermediate risk and 6 with high risk disease bagged in advance. Mutated IGHV genes (i.e. more than 2% of somatic mutations) were identified in 23 and unmaturated in 26 patients. Genetic abnormalities using fluorescence in situ hybridization (FISH) probes for del 13q, del 11q, del 17p and +12 were investigated in 40 patients. We divided patients according to genetic aberrations into favourable (no abnormalities or del 11q, n=25) and unfavourable group (del 11q or +12 or 17p or multiple abnormalities, n=15). Results. There was statistically significant increase of both VEGF (p=0.006) and bFGF (p=0.0001) in patients with B-CLL compared to the control group. Patients with mutated IGHV genes had significantly higher concentrations of bFGF (p=0.049) but not VEGF (p=0.146) than those with unmaturated IGK. Furthermore, bFGF was significantly higher in both IGHV subgroups (p=0.001) while VEGF was significantly elevated in IGK mutated (p=0.0002) but not unmaturated patients (p=0.0788). Regarding cytogenetics, significant difference between patients with favourable vs. unfavourable aberrations was nei-
promising in upfront therapy while the role of maintenance is still debated. Thalidomide (thal) is an active drug in the treatment of myeloma, and is been investigated as first line therapy. It could be useful in the control of minimal residual disease. We used thal as maintenance after autologous transplantation (single or double) and compare the outcome with other maintenance or none. From January 2001 to December 2005 25 patients (15 males and 12 females) with MM have been treated in our institution. Median age was 58 years (range 40-72). 12 were IgG, 7 IgA, 4 light chains and 2 plasma-cell leukaemia. Treatment was 4 cycles of VAD regimen followed by auto-SCT. 9/25 performed double auto-SCT. 3 months after SCT, 13 patients (9 single and 4 double SCT) began thal 50 mg/die as maintenance therapy. 12 patients (7 single and 5 double SCT) received IFN-50 mg/die as maintenance therapy, 12 patients (7 single and 5 double SCT) received IFN-50 mg/die and dexam 20 mg days gg 1-4 and 15-18. The difference between the 2 groups is statistically significant for PFS (p: 0.007), and not significant for OS (p: 0.057) even if difference (73% vs. 10% at 100 months) appears clear (Graph 3). From diagnosis the median OS is 72 months in no thal group and is 75% projected at 100 months (p: 0.2 graph 3) in the thal group. Thal was administered for a median period of 12 months, being neurological toxicity the main reason of suspension (3/10 patients). Neurological toxicity grade I-III was present in 65% of patients, while haematological toxicity grade I occur in 55% of patients. In conclusion, in a small number of patients low dose thal as maintenance after auto-SCT resulted in an improved PFS and OS when compared with other or none maintenance, with acceptable toxicity. Further studies in larger cohorts and randomized trials are needed to confirm this experience.
the clinical symptom manifestation. The only patient with intensive VEGF-R2 expression died within 8 months, this patient had the high levels of VEGF-A, VEGF2, VEGFR1 and VEGFR3 expression, as well. Two other patients, who died during 8-12 months of disease progression, also had the high levels of VEGFs and VEGFRs expression. The comparison of VEGFs and VEGFRs expression in patients before and after therapy revealed that VEGF-C expression was stimulated in patients resistant to therapy. Conclusions. Our data showed the high frequency and the similarity of VEGF-A and VEGF-C expression in bone marrow aspirates of MM patients studied. VEGF-D and VEGFR3 were expressed to the lesser extent. The high level of VEGFR1 expression was registered in all patients, while all patients except one were VEGFR2-negative. Our data suggest that VEGF-C expression could be the predictor of poor prognosis.

1409
UNUSUAL CNS AND CUTANEOUS INVOLvements IN MULTIPLE MYELOMA DURING BORTezOMIB TREATMENT
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Background. Since last years the proteasome has emerged as a real and exciting target for anticancer therapy. Velcade (bortezomib, PS341) remains the first selective proteasome inhibitor that has demonstrated significant preclinical activity in several tumor models and significant efficacy in patients with refractory or relapsed multiple myeloma. The major biological effect of bortezomib is the inhibition of the nuclear transcription factor NFkappaB, with subsequent inhibition of the tumor cells growth, induction of apoptosis, inhibition of angiogenesis and of cellular adhesion. In vitro bortezomib induces apoptosis of multiple myeloma cells and inhibits cell adhesion within the bone marrow microenvironment. The preliminary results of several phase I and II studies showed high antmyeloma activity of bortezomib alone or in combination with cytotoxic agents such as doxorubicin, melphalan, dexamethasone or thalidomide in patients with newly diagnosed multiple myeloma.

Figure 1. Patient 2. Cranial leptomeningeal myelomatosis.

Methods. We describe two cases of multiple myeloma patients that developed unusual cutaneous and CNS localizations during treatment with bortezomib alone. A 75 years old male patient with immunoglobulin G-kappa multiple myeloma resistant to previous therapies with melphalan and thalidomide received bortezomib (given day 1, 4, 8, 11 at 1.3 mg/m² every 21 days). After two courses a serologic response (from IgG 7170 mg/dl to IgG 1560 mg/dl) was obtained. However, several cutaneous lesions localized at the face, arms and chest were presented. The baseline evaluation revealed nodular lesions and skin thickening or localizations, therefore dexamethasone was added to bortezomib and a complete disappearance was observed 2 weeks later. A 74 years old female patient with immunoglobulin G-lambda multiple myeloma resistant to previous treatments with melphalan and thalidomide started a treatment with bortezomib (given day 1, 4, 8, 11 at 1.3 mg/m² every 21 days) because of disease progression (IgG 3400). After the first course of bortezomib, while the monoclonal component drastically reduced (IgG 1470 mg/dl), multiple sub-cutaneous nodular lesions and meningeal involvement of multiple myeloma with massive infiltration of cerebellum appeared and the patient died after one week. Conclusions. To our knowledge, these are the first cases of cutaneous and CNS localizations of multiple myeloma during treatment with bortezomib. Pharmacokinetic studies have demonstrated that after administration of a single dose bortezomib is rapidly distributed into nearly all tissues, with the exception of adipose tissue and certain tissues in the brain protected by the blood-brain barrier. Our cases show that bortezomib is fastly effective in reducing the size of the disease, but that it can’t pass the emato-encfallic barrier and can’t reach adipose tissue. Interestingly both patients were previously treated with thalidomide, a molecule that has been recently associated with extramedullary relapses probably because it increases the expression of cytoadhesion molecules such as CD158 and CD56 in myeloma cells. It could be of interest to evaluate in further studies the expression of cytoadhesion molecules also during treatment with bortezomib.

1410
COMBINED ADMINISTRATION OF BORTEZOMIB AND DEXAMETHASONE IN THE TREATMENT OF REFRACTORY MULTIPLE MYELOMA (MM)
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Multiple myeloma (MM) is a neoplastic disease especially affecting elderly patients even if in recent years it has been also observed in younger patients. The use of the proteasome inhibitor bortezomib has been recently introduced in the treatment of relapsed and/or refractory MM. In fact, Bortezomib has proven to be safe and effective in MM patients not only as monotherapy but also given in combination with cytotoxic agents. Bortezomib-based combination regimens have induced clinical benefits with manageable toxicities and may ultimately lead to improvement in the duration of response and survival of patients in the first-line setting. In our institution we are following 45 patients with stage II/III MM and 7 out of 35 (4 F and 3 M, median age: 71 years, r.: 68-77 years) suspended chemotherapy after 6 cycles of Melphalan and Prednisone regimen for excessive toxicity even if they presented progression disease (PD) at the clinical re-staging performed with both serum marker evaluation and cytological examination of bone marrow blood. All the 7 patients refused thalidomide treatment and underwent a treatment with bortezomib (1,3 mg/m² i.v. d. 1,4,8,11 every 21 days) together with dexamethasone (40 mg i.v. days 1-4 every 21 days). At a clinical re-staging performed after four courses from the beginning of bortezomib-dexamethasone combined administration a partial remission (reduction of M-component > 50-75%) was recorded in 6 out of 7 patients while the remaining was in steady disease (SD). Thereafter all patients received further four courses of therapy. At one month from the end of treatment two of seven patients achieved a complete remission (negative immunofixation) and the remaining showed a partial remission (PR). At the present, (month +9) only one patient shows a progression disease, while two patients are in CR and four in PR. Our results suggest that the combination of bortezomib and dexamethasone is effective and well tolerated in the treatment of elderly patients. Although there are several published data on the activity of the therapy based on the combination between bortezomib and dexamethasone, little is still known about the improvement in the duration of response and survival of elderly patients in the first or second line therapies.

1411
CLINICAL EFFICIENCY AND TOXICITY OF REGIMENS VAD AND HYPERCVAD IN MULTIPLE MYELOMA
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The primary objective of this investigation was to compare the overall response rates and toxicity of the standard regimen VAD-D-D and
regimen HyperCVAD for first line treatment patients with multiple myeloma. First group consist of 40 patients (57±3 years) which received 4-6 standard regimens VAD-D-D every 28 days. Second group consist of 20 patients (57±3 years) which received 4-6 standard regimens HyperCVAD (Cyclophosphamide 800 mg/m² i.v. twice 1-3 days, vincristine 2 mg i.v. day 1 and 9, doxorubicine 50 mg/m² i.v. day 4, dexametazone 40 mg per os 1-4 and 9-12 days) every 14-21 days. All patients had good performance status, elevated LDH values, but did not have low platelet count. An objective response (complete or partial) was documented in 45% and 65% of patients treated with VAD and HyperCVAD, respectively. Hematological and non-hematological toxicities were mild or moderate and equally distributed between the two treatment arms with the exception of neutropenia III-IV, which was more common after HyperCVAD (90%). The duration of neutropenia was from 5 to 9 days. 3 (15%) patients had febrile neutropenia. Early death was in first and second groups 5% and 0% respectively. Project 3 years overall survival was 65% and 90% in VAD and HyperCVAD groups respectively. These preliminary data suggest that regimen HyperCVAD more effective than standard regimen VAD.

### 1412 BORTEZOMIB AS SALVAGE TREATMENT IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA

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**Backgrounds.** Bortezomib is a proteasome inhibitor with significant antitumor activity, exerted through disruption of critical signaling cascades that promote cell adhesion and cycle progression, thus inducing apoptosis and decreasing cell proliferation. Bortezomib alone or in combination with dexamethasone has already been reported to yield high response rates and durable remissions in patients with relapsed/refractory multiple myeloma (MM). **Aim:** To evaluate the efficacy and safety of bortezomib as salvage treatment in our patients with relapsed/refractory MM. **Methods.** Between August 2004 and December 2005, 22 patients with progressive MM, 13 (59.1%) males and 9 (40.9%) females, of median age 65 (49-82) years, were treated with bortezomib. Thirteen (59.1%) patients were in relapse and 9 (40.9%) in refractory relapse. Median time from diagnosis was 22 (3-180) months. Patients had previously received a median number of 2 (1-4) different regimens and 5 (22.7%) had undergone high dose therapy with autologous stem cell transplantation. Bortezomib was administered subcutaneously at a dose of 1.3 mg/m² IV on days 1, 4, 8 and 11 every 2 weeks. In case of stable or progressive disease after 2 therapy cycles, dexamethasone 20 mg PO was added to the regimen on days 1-2, 4-5, 8-9 and 11-12 of each cycle. Response and toxicity were evaluated according to the Kaplan-Meier method. **Results.** Median follow-up time was 7 (2-15) months. Patients received a median number of 6 (1-8) therapy cycles. Median time to response was 2 (1-5) months. Complete response was observed in 2 (9.1%) patients, very good partial response in 4 (18.2%) and partial response in 11 (50%), yielding an overall response rate of 72.5%. Eight (36.4%) patients are dead and 14 (63.6%) are alive, 12 (85.7%) of which, in remission. Median and OS are not reached. Actuarial RD rate at 6 months and OS rate at 12 months was 57.6% and 52.5% respectively. Median EFS was 6 (95%CI: 1-15) months. Peripheral neuropathy was observed in 13 (59.1%) patients, thrombocytopenia in 11 (50%), fever in 9 (40.9%), microbial respiratory infections in 8 (36.4%), herpetic skin infections in 4 (18.2%), skin rash in 4 (18.2%), diarrhea in 3 (13.6%), nausea/vomiting in 3 (13.6%), hypotension in 2 (9.1%) and constipation in 2 (9.1%). Grade III-IV peripheral neuropathy, thrombocytopenia and respiratory infections were observed in 3 (13.6%), 2 (9.1%) and 3 (13.6%) patients respectively. A case of grand mal seizure during bortezomib infusion was noted. **Conclusion:** Bortezomib proved in our study to be an effective regimen in the treatment of relapsed/refractory MM, yielding very high response rates, while toxicity was acceptable. However, EFS was short, though a longer follow-up may be required in order to estimate patients' outcome more accurately.

### 1413 DECREASED γ-δ T CELL RECEPTOR (TCR γδ) EXPRESSION IN PERIPHERAL BLOOD LYMPHOCYTE POPULATION AND REDUCED SERUM OSTEOPROTEGERIN CONCENTRATION AS A TUMOUR ADVENTAGE MARKERS IN MULTIPLE MYELOMA (MM) PATIENTS

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Wrocław Medical University, Poland; WROCLAW, Poland

**Backgrounds.** γ-δ T lymphocytes (γδ T) appear to posses intrinsic cytolytic activity against tumour cells in carcinomas, sarcomas and lymphomas. OPG is known as a natural inhibitor of osteoclasts. **Aim:** to determine a mean percentage (%) of γδ T cells in peripheral blood and serum osteoproeregine (OPG) concentrations of untreated MM patients (pts) and verify the impact of peripheral blood γδ T cells presence and serum OPG levels at the time of diagnosis on MM clinical adventage. **Material and Methods.** 25 newly MM pts, admitted to Department of Hematology, Blood Neoplasms and Bone Marrow Transplantation of Wrocław Medical University between 2002-2005 were included into analysis. According to Case bone disease staging system: 6 pts were of 0 stage, 4 pt of 1, 3 of 2 and 12 of 3 stages. Samples of blood and sera were taken at the time of MM diagnosis. γδ T cells were estimated by flow-cytometry (FACS), using a fluorescein-activated cell sorter and monoclonal antibodies (MoAbs: Ab-anti TCRγδ-FITC (Becton-Dickinson), Ab-anti CD14-PE/CD-45-FITC (Leukogate) and CD3-PE. Serum OPG was estimated in enzyme-linked immunosorbent assay (ELISA, Biomedica GmbH, Wien, Austria). Results. In 12 of MM pts in 3 bone stage (group I) γδ T cells percentage, contained in interval 0,5-7,2 (mean = 2,75; SD = 1,9 ) was significantly (p=0,01) decreased as compared with 13 of MM pts in 0+1+2 bone disease stages (group II): 1,4 - 12,4 (mean=6,9, SD = 4,75). Despite a lack of statistical significance, the favorable trend was observed that serum osteoproeregine (OPG) concentrations in MM patients with abbreviated bone involvement (group I) fluctuated from 0,9 to 5,3 pmol/ml (mean = 2,54, SD = 1,47) and was lower than in MM pts with less advanced bone destruction (group I): 1,4 - 7,4 pmol/ml (mean = 3,68, SD = 2,07) (p=0,08). Moreover, positiive correlation between peripheral blood γδ T cell percentage at the time of diagnosis and serum OPG concentration was found: r = 0,48 (p= 0,08). **Conclusions.** In MM decreased γδ T cell percentage in peripheral blood and reduced serum osteoproeregine concentration, measured at the time of diagnosis seems to be advanced tumour markers in clinical practice.

### 1414 CARDIAC AMYLOID DEPOSITION AS A CAUSE OF MYOCARDIAL DYSFUNCTION IN MULTIPLE MYELOMA

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**Backgrounds.** Amyloidosis (AL) occurs in 10-20% of patients with multiple myeloma (MM). Around 40% of them manifest cardiac involvement on echocardiogram, in only half of which, overt congestive heart failure is observed at presentation. Doppler echocardiography best assesses myocardial function in AL by demonstrating infiltration or restriction during diastole. The precursor of B-type natriuretic peptide (pro-BNP) is a specific and sensitive biomarker secreted by the muscle cells of left ventricle (LV) in response to ventricular stress allowing early detection of myocardial dysfunction. **Aim.** To study the correlation between the serum osteoproeregine (OPG) concentration and the thickness of interventricular septum (IVS) and the degree of myocardial dysfunction, as translated by the value of E/A wave ratio on Doppler echocardiogram and the serum levels of pro-BNP. Methods. Twenty-six patients with multiple myeloma and no other medical condition that could affect the thickness of IVS or myocardial function, entered the study and were divided into Groups A and B according to the thickness of IVS (>14mm and ≤14 mm respectively). Eighteen healthy individuals with thickness of IVS <11mm, were used as control Group C. Doppler echocardiography was performed in order to estimate the thickness of IVS and E/A wave ratio and blood was drawn in order to measure pro-BNP serum levels. One-way NOVA tests were used to compare the values of E/A wave ratio and the measurements of pro-BNP serum levels between groups. Differences were assessed using the long-rank test. Results. Group A included 15 patients, 10 males and 5 females of medi-
Despite all the clinical experience gained since now, the patients of multiple myeloma and metastatic cancer treated in our institution all patients of multiple myeloma and metastatic cancer have been treated with intravenous bisphosphonates to evaluate the treatment outcome and formulate guidelines. Results of the UK MRC myeloma IX trial with regard to this complication in which patients with multiple myeloma are randomized to receive zoledronic acid or intravenous pamidronate will be interesting. We suggest that physicians and dental community should liaise closely with each other in the identification and management of this dreaded complication. We suggest patients should be informed of the risk of osteonecrosis. All patients should be reviewed by the oral surgeons before the start of bisphosphonates and any dental infections removed.

### Table 1. Showing patients profi.

<table>
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<th>Medications</th>
<th>No of infusions</th>
<th>Sign/symptoms</th>
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<td>Zoledronate</td>
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### 1415

**HIGH INCIDENCE OF OSTEONECROSIS OF JAW IN PATIENTS WITH MULTIPLE MYELOMA TREATED WITH ZOLEDRONIC ACID**

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**Background.** Bisphosphonates are of proven value in the prevention of skeletal-related events in patients with multiple myeloma and metastatic bone disease. In multiple myeloma these are used routinely in patients with skeletal lytic lesions. Several classes of bisphosphonates are used, however recently the use of zoledronic acid has increased due to its high potency in preventing bone mineral loss. This has however been associated with a new oral complication, osteonecrosis of jaw, in a proportion of patients with myeloma. The pathogenetic mechanism is thought to be bisphosphonate induced increased osteoclast activity and reduced blood flow in the bone which delays bone healing. We evaluated in our institution all patients of multiple myeloma and metastatic cancer who have been treated with intravenous bisphosphonates and looked for the incidence of osteonecrosis of jaw, underlying predisposing factors and outcome after management of these patients. Aims. To look for the incidence, clinical presentation, underlying predisposing factors for the development of osteonecrosis of jaw in patients with multiple myeloma and metastatic cancer treated with intravenous bisphosphonates. To evaluate the treatment outcome and formulate guidelines for the prevention of this complication. Methods. We evaluated all patients of multiple myeloma and metastatic cancer treated in our Institution with intravenous bisphosphonates for the occurrence of osteonecrosis of jaw. We looked at the type of bisphosphonate used, duration of use, concurrent use of chemotherapy, clinical presentation, precipitating factors and the treatment outcome. The suspected patients were evaluated and managed at the oral surgery department. Results. We found out of a total of 26 patients with multiple myeloma who were on intravenous bisphosphonates 6 developed pathologically proven osteonecrosis of jaw. Interestingly all six patients were females and all of them had received zoledronic acid. None of the 36 patients with non-haematological cancer who had received intravenous bisphosphonates developed osteonecrosis of jaw. The patient profile of the myeloma patients who developed osteonecrosis is given in table-1. Mean age of patients was 62 years. Median duration of onset of symptoms after the start of zoledronic acid was 48 months (range 24-65). Median number of cycles of bisphosphonate used was 44 monthly cycles (range 24-67). The commonest presentation was jaw pain, three patients had visible facial swelling. Two of the patients had non-healing sockets. Five patients were on concomitant chemotherapy (Thalidomide, cyclophosphamide, melphalan, interferon and steroids). Radiographs did show characteristic lytic lesions of multiple myeloma but as early inflammatory process is difficult to appreciate on x-rays only two patient films showed the typical healing response around the necrotic bone related to osteonecrosis of jaw. Biopsy of all patients was performed, tissues from non healing sockets showed florid lymphoplasmacytic infiltrate, histopathology of the exposed bony patches revealed abundant purulent material and necrotic bone and laboratory findings of the patient having soft swelling in the anterior arch were presence of hyperplastic fibroepithelial growth. Oro dental hygiene was poor in all patients. Only two patients had history of dental extraction. None of the patients had history of radiotherapy to head and neck. In all six patients zoledronic acid was stopped and was switched over to sodium clodronate. Debridement of non-healing sockets and exposed areas of bone were also carried out. Patients were given long term antibiotics and were encouraged towards maintenance of good oral hygiene and regular use of antimicrobial mouth washes. These measures brought substantive improvement to the quality of life of patients and relieved there symptoms to a certain extent however the lesions did not heal completely. After noticing these complications we discouraged the use of zoledronic acid outside the context of a clinical trial. Summary. Osteonecrosis of jaw following intravenous bisphosphonates has been noticed since 2003. Several case series have been published but as yet no proper guidelines for its prevention have been published. The incidence of jaw necrosis in patients with myeloma in most published series is about 4%. Majority of cases occur following zoledronic acid (80%) however pamidronate and even alendronate have been implicated. Dental procedures, poor oro dental hygiene, radiotherapy and in some cases concomitant use of anti-angiogenic agents like thalidomide or corticosteroids are thought to be the commonest predisposing factors. In our short series of cases, we found a high incidence of (6/26) this complication in patients with multiple myeloma. This is worrying for the patients and treating physicians. Large case studies are warranted to delineate the predisposing factors and formulate management guidelines. Results of the UK MRC myeloma IX trial with regard to this complication in which patients with multiple myeloma are randomized to receive zoledronic acid or intravenous pamidronate will be interesting. We suggest that physicians and dental community should liaise closely with each other in the identification and management of this dreaded complication. We suggest patients be informed of the risk of osteonecrosis. All patients should be reviewed by the oral surgeons before the start of bisphosphonates and any dental infections removed.
tual units that sent us patients with MM suspicion, the stage of the disease. We had 56% men, 44% women. Results: 88% of MM patients, 8% of Waldenstrom disease, 7% of solitary plasmocytoma and 5% of MGUS. Biological modifications were: increased ESR 15%, anaemia 15%, hypergammaglobulinemia 8%, hyperproteinaemia 1.5%, monoclonal migration 1.5%. The most important clinical sign was the bone syndrome (bone pain). Bone lises were localised on: the skull 48%, rib cage 28%, pelvis 18%, lumbar spine 6%. Neurological manifestation were 23% progressive radicular pain and 77% sensory or motor defects. General signs (asthenia, dis ease, low effort capability) were present in 88% of the cases and infections in 50% of the cases. The profile of the clinics that contributed to the identification of the disease was: 31% internal medicine, 10% gastroenterology, 11% nephrology, 10% neurosurgery, 5% cardiology, 5% diálisis, 5% pneumohipotisology, 3% chest surgery, 3% neurology, 3% urology, 2% reumatology, 2% plastic surgery, 2% general surgery, 2% oncological surgery, 2% oncology, 2% gynecology, 2% ORL. At diagnosis patients were in stage I 11%, stage II 16%, stage III 73%. Borrowed diagnosis were: anaemia 17%, lumbar disorder 11%, toracic tumor 11%, monoclonal gamapathy 6%, chronic renal failure 6%, radiculonevritis 6%, reumatic disorder 4%, fronto-parietal tumor 4%, colagenosis 2%, atrosis of the cervicodorsolumbar spine 2%, gonnartesis 2%, superior digestive haemorrage 2%, medulary hypoplasia 2%, digestive neoplasia 2%, hepatoplastic sindrom 2%, Raynart sindrom 2%, fever sindrom 2%, pulmonary no sinus 2%, pulmonary sinosis 2%. What is really important in the results of our study is that 74% of the patients with MM suspicion came from other hospital units not from the primary medicine units and also the percent of patients in stage II at diagnosis was 73%. Conclusions. 73% of patients were stage III at first presentation which means that there are some problems to recognise and supervise this disease at primary medicine units. There are some biological.test that remain unused for the patients which could contribute at early diagnosis of MM. Funding: Study supported by grant TD 22 no.27690/14.03.2005 of CNCSTS (Romanian National Council of Scientific Research)

1417
SERUM-ASCITES ALBUMIN GRADIENT DIFFERENTIATES TWO TYPES OF ASCITES IN MULTIPLE MYELOMA: REPORT OF TWO CASES AND REVIEW OF THE LITERATURE
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tology, WARSAW, Poland

Ascites is extremely rarely encountered in multiple myeloma (MM), either at presentation, or in the course of the disease. To diagnose myelomatous ascites tumor plasma cells and/or monoclonal protein must be demonstrated in peritoneal fluid. However, a detailed characteristics of myelomatous ascites as a clue to its pathogenesis has not been given. To reveal characteristics of ascitic fluid in myelomatous ascites we report two cases of MM patients, one with ascites and reviewed the pertinent literature. MM has been diagnosed on the International Myeloma Working Group criteria (Br. J. Haematol. 2003;121:749-757) and staged by Durie and Salmon (Cancer 1975;36:842-854). Peritoneal fluid samples have been assessed according to Runyon’s guidelines (Hepatology 2004;39:1-16). We determined ascitic total protein, M protein, and albumin, serum-ascites albumin gradient (SAAG), ascites to serum total protein and lactate dehy-
drogenase ratios, total nuclear cell count and differential, culture and cytology. A chylous ascites has been confirmed by ascitic triglycerides level >200 mg/dL. From English and French full text articles searched out by Medline all cases of MM-related ascites with an adequate ascitic fluid data have been chosen for analysis. We assumed that at least ascitic protein, total nuclear cell and/or plasma cell count, and given or calculable SAAG must have been available. The nonparametric Mann-Whitney test has been applied. From January 2004, we managed 2 patients with MM presenting as ascites (a 91-yr-old male with IgA type at IIIA stage and a 72-yr-old female with IgG type at IB stage). In both patients ascites was chylous, had characteristics of an effusion, high SAAG, and contained tumor plasma cells. At first, our patients were treated with weekly dexamethasone, then received melphalan and prednisone course, but survived only 1 and 3 months, respectively, from a diagnosis of ascites. Of 34 cases of MM-related ascites reported during the last 40 years, we could choose only 9 additional patients who had an adequate ascitic fluid characteristics. On the whole, we analyzed 11 cases (Table) and revealed two types of myelomatous ascites with low (<1.1 g/dL) and high (≥1.1 g/dL) SAAG. In the former a median ascitic total nuclear cell count and plasma cell count were higher than in the latter, 4100/µL (range 1000-9000) and 340/µL (range 40-800) (p=0.008), and 3081/µL (range 1000-8550) and 120/µL (range 30-450) (p=0.016), respectively. Three of 11 cases had a chylous ascites.

Table 1.

<table>
<thead>
<tr>
<th>Year</th>
<th>Age (yrs)</th>
<th>Type</th>
<th>Sex</th>
<th>Color</th>
<th>M band</th>
<th>Protein</th>
<th>SAAG</th>
<th>Cell</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poth/1971</td>
<td>45/F</td>
<td>bloody</td>
<td>NA</td>
<td>6.4</td>
<td>0.4</td>
<td>9000</td>
<td>8550</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thomas/1973</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>4.5</td>
<td>&gt;1.1</td>
<td>150</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High/1975</td>
<td>76/M</td>
<td>chylous</td>
<td>2.5</td>
<td>5.9</td>
<td>1.0</td>
<td>NA</td>
<td>1000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greer/1985</td>
<td>57/M</td>
<td>bloody</td>
<td>4.6</td>
<td>7.1</td>
<td>&lt;1.1</td>
<td>4100</td>
<td>1640</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gorg/1988</td>
<td>44/M</td>
<td>bloody</td>
<td>1.5</td>
<td>5.8</td>
<td>&lt;1.1</td>
<td>1000</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Akery/1999</td>
<td>51/F</td>
<td>yellow</td>
<td>+</td>
<td>2.4</td>
<td>&gt;1.1</td>
<td>40</td>
<td>37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kenen/1999</td>
<td>71/F</td>
<td>yellow</td>
<td>2.0</td>
<td>0.9</td>
<td>6600</td>
<td>4422</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singh/2004</td>
<td>45/M</td>
<td>GA</td>
<td>NA</td>
<td>&gt;2.0</td>
<td>&gt;1.1</td>
<td>340</td>
<td>136</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoue/2005</td>
<td>51/F</td>
<td>yellow</td>
<td>1.8</td>
<td>2.9</td>
<td>0.8</td>
<td>2220</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sikorski/2006/91</td>
<td>M</td>
<td>bloody</td>
<td>3.2</td>
<td>6.9</td>
<td>1.7</td>
<td>600</td>
<td>450</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sikorski/2006/72</td>
<td>F</td>
<td>chylous</td>
<td>1.3</td>
<td>3.6</td>
<td>1.6</td>
<td>800</td>
<td>120</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NA, not available data; F, female; M, male.
requires alertness and prompt management. Rigorous oral hygiene and avoiding extensive dental procedures in patients who receive bisphosphonates could assist in preventing this complication.

**Figure 1.** ONJ in a bisphosphonate-treated MM patient.

### 1419

**OSTEONECROSIS OF THE JAW IN MULTIPLE MYELOMA PATIENTS: MONOCENTRIC EXPERIENCE**

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Multiple myeloma (MM) is a common lymphoproliferative disorder. Modern therapies have remarkably improved the survival of affected patients. Thus, long term adverse events related to chemotherapy and/or ancillary treatments are observed with increasing frequency. Bisphosphonates (BP) are synthetic analogues of pyrophosphate. These compounds have been approved for treatment of cancer-related hypercalcemia and bone lytic involvement by MM and solid tumors. Zoledronic acid (ZA) is the most potent BP. It has antiangiogenetic activity and inhibits osteoclastic differentiation and normal bone turnover. Recent-ly, several cases have been reported of avascular osteonecrosis of the jaw (ONJ) associated with the use of ZA in patients with MM. Jaw is the only bone exposed to the outside and often site of traumatisms which induce osteoclasts activation. The objective of this monocentric analysis consists in the retrospective evaluation of the effects of treatment duration with ZA on the onset of ONJ in a cohort of 64 patients which were autotransplanted at our Institution. ONJ was evaluated by craniofacial complex CT and/or MRI followed by tissue biopsy for pathological and microbiological exams. Among the 64 analyzed patients, 5 (3 in long lasting complete remission and 2 in very good partial remission) developed ONJ. Two of this also showed a massive necrosis of both maxillary sinuses. All of the patients referred pain and swelling, two of them also referred purulent discharge and necrotic jawbone exposure. ONJ was documented by biopsy in three of the five patients (3 men and 2 women). BP therapy was discontinued in all cases. Three patients underwent surgical curettage and all five were treated with antibiotic therapy. The outcome has been resolution of necrosis in three patients, persistence of bone exposure in one patient and oral antalin communication and cutaneous fistula in the other. Time to jaw osteonecrosis diagnosis since the beginning of BP treatment was three years in one patient, 4 years in two patients and 7 years in the last two. Osteonecrosis of the jaw in patients with MM can be associated with BP therapy. BP mechanism of action, that includes osteoclasts apoptosis and antiangiogenetic effect, is responsible for reduction of local blood flow and retard in bone repair. This leads to jaw bone damage. Duration of therapy with ZA is crucial in the development of this complication in affected patients.

### Table 1. Table of FLC levels in serum and dialysis.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dialysis</th>
<th>FLC type</th>
<th>Mean blood pre (mg/L)</th>
<th>Mean blood post (mg/L)</th>
<th>Mean% Removed</th>
<th>Total in dialysate fluid (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>λ</td>
<td>10626</td>
<td>4310</td>
<td>59.6</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>λ</td>
<td>9155</td>
<td>3760</td>
<td>58.6</td>
<td>31</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>λ</td>
<td>3362</td>
<td>2445</td>
<td>23.7</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>κ</td>
<td>2758</td>
<td>1489</td>
<td>45.9</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>κ</td>
<td>861</td>
<td>329</td>
<td>61.8</td>
<td>19.6</td>
</tr>
</tbody>
</table>

### 1421

**QUANTITATIVE BONE ULTRASONOGRAPHY AT PHALANGES IN PATIENTS WITH PLASMA CELL DISCRASIAS AND ZOMETA TREATMENT**

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The current aims of bisphosphonates for metastatic bone disease are to prevent skeletal-related events (SREs), reduce bone pain and improve quality of life. Zoledronic acid (Zometa) is the most potent tested bis-
phosphonate. Additional studies are needed to determine the optimal timing, schedule, and duration of treatment in myeloma patients. DBM Sonic Bone Profiler is the only ultrasound device that applies the method of signal analysis in transmission through phalanges. This technique of clinical investigation has proven to be particularly effective in postmenopausal osteoporosis for screening, diagnosis and therapies monitoring. To assess Bone Mineral Density (BMD) in patients with plasma cell dyscrasias along the time of Zometa™ treatment using quantitative ultrasound analysis in transmission through phalanges. 12 pts with MGCUS (3 IgGk, 4 IgGλ) and 4 pts with myeloma IIIA (3 IgGκ, 1 IgA lambda), M/F=6/10, median age 69 yrs received zoledronic acid 4 mg intravenously every 4 weeks for at least 12 courses. All subjects took daily oral supplements containing elemental calcium (1 g) and vitamin D (400 IU). Myeloma patients also received chemotherapy. Measurements of bone mineral density were performed by ultrasonography at phalanges at baseline and at 1, 3, 6, 9, 12, 15 and 18 months. The ultrasound signal is analysed to generate the test report parameters, AD-SoS (Amplitude Dependent Speed of Sound), UBPI (Ultrasound Bone Profile Index), BTT (Bone Transmission Time) that express the properties of density and structure of bone tissue. As showed in the picture all patients improved AD-SoS together with all measured parameters (UBPI and PTT) starting from 4 to 16 month (p<0.005) in comparison with baseline values. Densitometric-structural evaluation of bone tissue at distal metapysis of the first phalanx of the II, III, IV and V hand finger is safe and useful to evaluate bone quality change during Zometa treatment.

1422 WISKOTT-ALDRICH SYNDROME SHOULD MEDIUM PLAQUETARY VOLUME (MPV) MATTER?
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Background and Aims. Wiskott-Aldrich syndrome (WAS) is typically X-linked and is characterized by a clinical triad: eczema, thrombocytopenia with small platelets and immunodeficiency (predisposing to autoimmune phenomena, lymphoproliferative disease and neoplasia). However, there can be polymorphism on several levels. In transmission autosomic dominant, transcription cis or trans of the mutated gene 1 or 2 is hospitalized with bloody diarrhoea, petechiae on abdominal wall and hemorrhagic component, with blood crusts, spreading from the face, neck, axila, dorsal and inguinal regions; hepatosplenomegaly has grown almost to iliac crest. He is medicated with co-trimoxazole and azithromycin (prophylaxis), folic acid and monthly paclitaxel. It was done a complete immunologic study at 6/7 months old, that revealed: hypergglobulinemia with hyperIgM (without deficit of IgG or IgA); lymphopenia T CD8⁺; elevated expression of activation markers on T lymphocytes, however without proliferation after in vitro stimulation; NK lymphocytosis. Clearly having evidence of a primary or secondary T immunodeficiency, it is made a search for mutations ZAP-70 and WASP. At 8 months old he is hospitalized by undetermined feverish syndrome and develops a very severe autoimmune hemolysis with shock that lead to his death. The confirmation arrives a mutation in exon 10 of WASP gene. Conclusions. In a case with an immunodeficiency this severe, briefly one has to think in bone marrow transplantation (the family was already being HLA typed). WASP protein seems to be involved in mechanisms of signal transduction between surface receptors and cytokine, provoking defects of chemotaxis, fagocytosis and presentation of antigens to T cells with inappropriate response. In platelets, there are diminished surface glycoproteins and adhesion defects. In literature, we find more and more heterogeneous cases, whether in genetic/phenotypic expression or clinical expression (as platelets with normal MPV), that can help achieving a better understanding upon mechanisms of WAS.

1423 THROMBOTIC THROMBOCYTOPENIC PURPURA: REPORT OF 27 CASES
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Thrombotic thrombocytopenic purpura (TTP) is a severe multisystemic disorder characterized by microangiopathic hemolytic anemia, thrombocytopenia, fever, fluctuating neurological symptoms and impaired renal function. Aims. To present the experience of the Department of Hematology, Clinical Center, Skopje, regarding this issue. Patients and Methods. Our study includes 27 patients (pts) with TTP between 1986 and 2006. All patients had microangiopathic hemolytic anemia and thrombocytopenia. There were 14 women and 13 men; the median age was 36 years (range 20-71). It doesn't appear to be related to other diseases. Results. The mean period between the first symptoms and the diagnosis was 8.5 days (range 1-21). Neurological symptoms were present in 21 patient, bleeding in 19, fever in 6 and renal impairment in 35. Median hemoglobin was 65 g/L (range 20-120) and median platelet count was 49x10¹²/L (range 6-130); median reticulocytosis was 6% (range 1-25). Results of screening tests of coagulation showed elevation of EDF in 7 pts. Serum LDH was increased in all patients - median 1748 IU (range 618-4520). Treatment included: corticosteroids in all patients, exchange plasmapheresis (EP) in 12 pts, only plasma infusions in 13 pts, antiplatelet agents in 10 pts. Plasma exchange is currently not available in our country. In one patient with exacerbation during the first TTP episode treatment with vincristine was introduced. There were 7 complete responders (5 on EP) and 14 deaths (3 on EP). Among the survivors 6 pts relapsed (2 pts had 2 relapses), 2 of them died during the first relapse. The median time delay from the onset of symptoms and treatment initiation lasted for 8.5 days (range 1-21), indicating poor disease recognition. The median time delay from diagnosis to EP was 5.5 days (1-11) suggesting relatively good EP availability. The median treatment duration in all patients was 15.5 days (range 1-40). The median number of EP cycles needed for the platelet stabilization was 4 (range 2-10). Conclusion. TTP is a severe disorder necessitating early recognition and diagnosis which would lead to treatment with EP on time. EP improves survival dramatically.

1424 A CORRELATION STUDY BETWEEN SERUM POTASSIUM LEVEL AND AETIOLOGY OF THROMBOCYTOSIS.
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Pseudo-hyperkalaemia is a rarely encountered event among patients with thrombocytosis. Typically, a raised serum potassium level is observed in the absence of renal failure. Occasionally, samples are reported to be haemolisated and results discounted. The interpretation of hyperkalaemia in the presence of renal failure (raised urea & creatinine) is problematic and causes anxiety among clinicians signing the results. The association between pseudo-hyperkalaemia and reactive causes of thrombocytosis is five-year audit and the presentation of papers with thrombocytosis who were referred to the Haematology Department in Ulster Hospital Dundonald. Seventy-one patients were identified from the ward registry. Clinical and laboratory data was obtained following chart review and entered into SPSS version 10 software package. A predominately elderly patient population is treated in this district general hospital. Twenty percent of patients had primary thrombocytosis; two thirds were essential thrombocytopenia. The most frequent causes of secondary thrombocytosis were iron deficiency, infection, malignancy and chronic inflammatory diseases. Raised serum potassium was more likely to be noted when platelet counts exceed 800 in myeloproliferative disorders especially primary thrombocythaemia; it was rare among patients with reactive causes of thrombocytosis. A weak correlation was observed between platelet count and serum potassium levels. Pseudo-hyperkalaemia led to patient admission to hospital, administration of calcium resorcin, 5% dextrose / insulin, and introduction of low-potassium diet in several patients in an attempt to lower serum potassium level. Failure to correct pseudo-hyperkalaemia resulted in serial venepunctures for U+Es and repeated dosing of potassium-lowering measures. All cases of pseudo-hyperkalaemia were detected by haematologists consulted regarding the aetiology of thrombocytosis. The falsely raised potassium level is due to release of intracellular potassium from the platelets during formation of clot in the 'gel' clotted specimen. This is a time-dependent phenomenon; the use of plasma sample in either Li-heparin or Na-heparin bottles will circumvent this phenomenon.

1425

EFFECT OF VASOPRESSIN AND ITS ANALOG DGAVP ON PLATELET AGGREGATION

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It is well known that neuro-hypophyseal peptide vasopressin (AVP) causes blood coagulation due to increased secretion of tissue thromboplastin and factor VIII:C into blood. Besides AVP exerts significant influence on platelet aggregation through interaction with specific receptors. AVP synthetic analog desglycylamid-arginine vasopressin (DGAVP) lacks peripheral hormonal activity and evokes blood procoagulant activity too. The aim of this study was to compare effect of AVP and its analog DGAVP as inductor of man or rat platelet aggregation. Effect of AVP and DGAVP as inductor of platelet aggregation was studied on platelet-rich blood plasma (PRP) in the children without bleeding disorders or in experiment on white rats. Platelet aggregation was induced by adding the peptides (in final concentration 10-5 M and 10-6 M) to PRP. Our results demonstrate that as AVP it’s analog DGAVP induced platelet aggregation in children PRP and effect of DGAVP was more intensive than AVP effect (degree of aggregation was 45% and 55% and rate of aggregation - 15,5% and 20% accordingly). But in experiment on rat we showed that these peptides induced more weak platelet aggregation (degree of aggregation was only 14% - 16,5%). However in this case DGAVP administration lead to more intensive platelet aggregation too. Besides DGAVP lead to reestablishment of platelet ADP-aggregation in children who had got platelet aggregation disorders with ADF. Thus we conclude that AVP analog DGAVP as natural factor induces platelet aggregation in man or rat PRP but it’s effect is more intensive. Besides our results demonstrate that as DGAVP as AVP effect on platelet aggregation can be both indirect and direct.

1426

TREATMENT OF IMUNE THROMBOCYTOPENIC PURPURA. POST-SPLENECTOMY LATE RESPONSE

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Backgrounds. Immune thrombocytopenic purpura (ITP) is a chronic disease with a good response to corticosteroids which is used as first line therapy. Some times after steroid therapy patients relapse and in this case splenectomy in the second line of therapy. Aims: We tried to evaluate the therapeutic results in a group of patients with ITP and the duration of their remission after splenectomy. Methods: From may 1990 - may 2000 we were hospitalised and treated in the Hematology Clinic 135 patients with ITP. Median age was 48 years with a distribution on sexes: 46 males and 59 females. Our patients 59% presented gastrointestin al bleedings, 52% had sclerogtometral bleedings and 9% had bleedings in the central nervous system. The most of the patients 91% were treated with corticosteroids, 12% received steroids and immunoglobulins. 35% (47 patients) had a splenectomy because they relapsed after steroids or they needed very high doses of corticosteroids for a safe number of trombocytes. From those 47 patients with splenectomy 19 were males and 28 were females with a median age at the time of splenectomy of 38 years. The medium time from diagnosis to splenec tomy was 3,5 years (0,6-96 months) The response to splenectomy was defined as follows: complete response (CR) a number of trombocytes higher than 150.000/mms for more than 4 weeks, partial response (PR) trombocytes between 50.000-150.000/mms lasting more than 4 weeks and relapse a number of trombocytes under 50.000/mms. Results. The medium follow-up time was 7 years (2-10 years). The overall response was 79% with 58% of CR and 21% PR. From 47 patients with splenectomy 15 patients relapsed and 5 of this 15 were in CR after steroid therapy following splenectomy. The long term follow-up in CR and PR proves a good, stable and durable response in time for more than 7 years. Post splenectomy complications in the study group were not significant. Conclusions. Our study proves that patients with chronic immune thrombocytopenic purpura who failed corticotherapy get a safe and durable response in time after splenectomy.

1427

INADEQUATE RESPONSE TO RITUXIMAB IN PATIENTS WITH CHRONIC REFRACTORY IMMUNE THROMBOCYTOPENIA (ITP)

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Background. Immune Thrombocytopenia is an autoimmune disease which involves opsonisation of platelets by auto-antibodies directed against different surface glycoproteins, leading to their premature destruction, in the reticulo-endothelial system. Recent studies suggests that Rituximab, a chimeric monoclonal antibody (against CD 20 B cells) is useful in the treatment of chronic refractory ITP. However, in comparison, at our centre, the response to Rituximab has been poor. Aims. Retrospective review of platelet counts (over a period of 6 months) of 4 patients treated with Rituximab.

Table 1. Rituximab in Chronic Refractory ITP.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age</th>
<th>Sex</th>
<th>Co-morbidity</th>
<th>Treatment Before Rituximab</th>
<th>Platelet Count Pre-Rituximab</th>
<th>Platelet Count Post Rituximab</th>
<th>Treatment During &amp; After Rituximab</th>
<th>Response</th>
<th>Time to Maximum Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35 y / f</td>
<td>Nil</td>
<td>Ig</td>
<td>Steroids</td>
<td>36</td>
<td>40</td>
<td>Ig Anti-D</td>
<td>4 wk</td>
<td>Minimal Response</td>
</tr>
<tr>
<td>2</td>
<td>50 y / M</td>
<td>NIDDM Hypercholesterolaemia</td>
<td>Steroids</td>
<td>69</td>
<td>70</td>
<td>Nil</td>
<td>RA</td>
<td>Not Response Applicable (NA)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>68 y / F</td>
<td>Osteoarthritis</td>
<td>Ig</td>
<td>Anti-D Danazol</td>
<td>34</td>
<td>20</td>
<td>Ig</td>
<td>NA</td>
<td>Response</td>
</tr>
<tr>
<td>4</td>
<td>80 y / F</td>
<td>Nil</td>
<td>Ig</td>
<td>Steroids</td>
<td>29</td>
<td>28</td>
<td>Splenectomy</td>
<td>NA</td>
<td>Response</td>
</tr>
</tbody>
</table>

Methods. We have used 4 cycles of Rituximab (375 mg/m²) administered at weekly intervals to 4 patients (3 females,1 male; mean age 58 years) with chronic refractory ITP. All 4 patients had previous treatment with immunoglobulins. Three patients had had prior treatment with Prednisolone; one did not receive Prednisolone because he was a Diabetic. One out the four patients had also received prior treatment with Anti D and Danazol (along with Immunoglobulins and steroids).
Of the 4 patients 1 had a Minor Response and 3 had No Response. However Rituximab was well tolerated in all 4 cases with no major side-effects. Conclusions. Our results suggest that Rituximab hardly made any impact on the platelet count of these 4 patients with chronic refractory ITP. Previous studies of Rituximab in ITP has shown an overall response rate of around 50%. However such initial results must be considered in the light of positive report bias, small numbers, lack of long term follow up and lack of randomised controlled trials. In addition, data on short and long term side-effects of Rituximab are lacking. Thus, Rituximab is an unproven treatment for chronic refractory ITP. Perhaps novel agents like Thrombopoietin Receptor Agonists should be considered for these patients with chronic ITP, in the setting of a Clinical Trial.

1428
EFFECTS OF VARIOUS THERAPEUTIC REGIMENS ON PLATELET FUNCTIONS IN PATIENTS WITH MYELOPROLIFERATIVE DISORDERS

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Blinding and thrombosis are common causes of morbidity and mortality in patients with myeloproliferative disorders (MPD). Qualitative platelet abnormalities are frequently found in these patients and range from platelet hyperfunction, acquired storage pool disease and/or platelet membrane defects, to abnormalities suggesting increased platelet reactivity, increased plasma β thromboglobulin levels or shortened platelet survival. In the present study, we aimed to investigate platelet function abnormalities using both optical platelet aggregometry and whole blood platelet aggregometry and evaluate the effects of various therapeutic regimes on these abnormalities, in patients with MPD. 45 patients with newly diagnosed chronic myeloproliferative disorders (26 CML, 11 PCRV, 8 ET) were enrolled. Median age was 54; there were 23 females and 22 males. At the study entry, whole blood count, PT, aPTT, fibrinogen, platelet aggregation studies by luminescence method in whole blood and by optical method in PRP, ristocetin cofactor activity were performed. The agonists used were; ADP, Arachidonic acid (AA), Ristocetin and Collagen. Platelets were considered to be hyperactive if at least one result (aggregation or ATP release with one agonist) was above the reference range, and hyporactive if at least one result (aggregation or ATP release with one agonist) was below the reference range. Mixed hyper- and hyporactive platelets were considered present when at least one result (aggregation or ATP release) was below and above the reference range, respectively. Repeat platelet function studies were performed in 20 patients, following specific therapy regimes. By luminescence method; before therapy 15/45 patients had platelet hyperfunction, 17/45 patients had coexistence of hyper- and hypofunction and 12/45 patients had platelet hypofunction. 1/45 patient had a normal result. After therapy 13/20 patients had platelet hypofunction, 2/20 patients had platelet hyperfunction, 2/20 patients had coexistence of hyper- and hypofunction while 3/20 patients had normal results. By optical method; before therapy 18/45 patients had platelet hyperfunction, 9/45 patients had platelet hyper- and hypofunction, 7/45 patients had platelet hypofunction whilst 1/11 had normal results. After therapy 15/20 patients had coexistence of hyper- and hypofunction, 4/20 patients had platelet hyperfunction, 1/20 patients had platelet hypofunction while none of the patients had normal results. We conclude that; 1. Different platelet function defects are present in most of patients with MPD. 2. Patients with CML have platelet hyperaggregability while patients with PCRV and ET have platelet hypoaggregability. 3. Our observations highlight the need to use WBPA to select patients for antiplatelet therapy in MPD. 4. Luminescence method appears to be more sensitive than optical method to evaluate platelet functions.

1429
ADIPONECtin ADDED INTO THE PLASMA OF HEALTHY PROBANDS DOES NOT AFFECT PLATELET AGGREGIBILITY

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Background. Adiponectin exhibits important antidiabetic and antiatherogenic effects. Although hypoadiponectinemia is associated with obesity-related metabolic and vascular diseases, the role of adiponectin in thrombosis remains elusive. Recent paper informed that adiponectin deficiency in adiponectin knockout male mice leads to enhanced thrombus formation and platelet aggregation. Aims. Evaluate of added adiponectin effect into the plasma in platelet aggregability. Methods. 6 healthy nonobese healthy probands were tested. In all of them platelet aggregability and adiponectin values were measured. Human adiponectin (Biovendor, Czech Republic) was added to PRP in different concentrations (100, 75, 50 and 25 ng/l). Than PRP was 5 min incubated and was evaluated induced platelet aggregation using CPG (Analytical Control Systems) at 3 µmol/l as the final concentration of CPG added to PRP with an Apact II platelet aggregometer (Labitec GmbH). Induced aggregation extent was defined by the slope of aggregation curve. Results. Adiponectin values had normal distribution in tested group (15.7-15.8 ng/l). Neither of tested probands had significant difference of the slope CPG values, even if 100 ng/l adiponectin concentration was added. Conclusions. The present study did not verify hypothesis about the in vitro human hyperadiponectinemia as an antithrombotic factor. Adiponectin concentration about 10 ng/l have similar antithrombotic in vitro action as values upper 100 ng/l.

1430
PRESENTATION OF NEW METHOD FOR ASA RESISTANCE DETECTION

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Background. Aspirin resistance seems to be an important prognostic factor in patients with coronary artery disease, but there is limited data on its correlation to clinical outcomes. Various methods for both in vivo and in vitro platelet function exist. In late 1990s, a novel in vitro inducer of platelet aggregation - cationic propyl gallate (CPG) was introduced into clinical practice, announced as an unprecedented, highly sensitive and specific method for assessment of aspirin resistance. In classic aggregometry problem with patients compliance remain unresolved. Recently there were present information about chance for ASA resistance testing by virtue of in vitro aggregation test with ASA addition. Aims. evaluate platelet ASA resistance with platelet CPG aggregation after ASA addition.

Methods. 20 healthy individuals and 20 patients with metabolic syndrome were evaluated. No individuals were ASA treated. In all of probands was performed platelet aggregation (Multiplate) after CPG induction. In part of whole blood was supplement solution of ASA (Aspisol, Bayer) and was perform aggregometry, over again. Results. Healthy probands have higher difference between AUC before and after the ASA pretreatment. (p<0,01, Kruskal Wallis) than probands with metabolic syndrome. CPG have higher difference before collagen (p<0,05). AUC of aggregometry line in all of healthy probands had significant reaction after Aspisol addition. On the contrary, AUC of patients with metabolic syndrome reacted different. Conclusion: authors presented frequent ASA resistance existence in individuals with metabolic syndrome against healthy. At the same time presented new in vitro method for ASA resistance detection which eliminace patient non compliance errors.
**1431**

**ASPIRIN STRENGTHENS ANTITHROMBOTIC EFFECT OF HEPARIN-LIKE ANTICOAGULANT FROM PLANT**

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**Background.** Effective antithrombotic agents possessing antiplatelet activity, are preparations such as aspirin, dipiridamol and others. Low molecular weight heparin also shows antithrombotic activity. Antithrombotic effect of plant heparin-like anticoagulant from roots of a peony at chronic intranasal administration has been shown. Aims. The purpose of the work was to study antithrombotic effects of plant heparin (PH) from Paeonia suffruticosa with antiplatelet aspirin at simultaneous peroral application of these preparations. Methods. In the work methods electrophoretic, chromatographic and spectral analysis were used. The formation of blood clots was carried out on method Wessler with our updating - in isolated by metal clips a fragment vein jugular entered thrombin with activity 3-5 sec (thrombin+stas of vessel). PH (1mg/mL) and aspirin (1 mg/mL) mixed in the ratio 1 : 1 (w/w). This mix was administrated peroral to animals (albino rats) in daily volume 0.5 ml/kg of body weight within 7 days before formation of blood clots. Antithrombotic effect was estimated on frequency of cases of thrombus formation and on weight of blood clots after 1 hour after thrombus formation. Results. At formation of blood clots of animals on a background of action PH + aspirin thrombus either were not formed (did not come to light) or were small (in case of their formation). So, quantity of cases of thrombus formation in experiment on a background administrated PH + aspirin made 58 per cent from the control = 100 per cent (administered PH + aspirin made 8 per cent, on a background administrated PH alone thrombus either were not formed or were small). PH and aspirin used in the work together with PH has shown higher antithrombotic effect against one PH. The difference in antithrombotic effectiveness between PH + aspirin and PH was 10 -11 per cent. So, we have established, that aspirin strengthens antithrombotic effect of low-molecular plant heparin.

**1432**

**SUCCESSFUL TREATMENT OF HEPARIN INDUCED THROMBOCTOPENIA TYPE II AND THROMBOSIS WITH FONDAPARINUX IN A DIALYSIS PATIENT**

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Although rare heparin induced thrombocytopenia (HIT) type II is one of the most feared complications of heparin therapy. Unfractionated heparin as standard anticoagulation used in hemodialysis sessions. Dialysis patients who are continually exposed to heparin are at risk for HIT. We report a 75-year-old male patient with acute-on-chronic renal failure who subsequently developed HIT while on hemodialysis. The patient admitted to our emergency department with dyspnea, tachypnea and bilateral low lung fields. His physical examination and laboratory assessment revealed acute lung edema, uric acidosis and acute-on-chronic renal failure requiring urgent renal replacement therapy. His history revealed hypertension for 15 years and diabetes for 1 year. A double lumen hemodialysis catheter was inserted into the left femoral vein and regular hemodialysis therapy with unfractionated heparin as anticoagulant was started. 10 days after starting hemodialysis the platelet count dropped from 504000/mm³ to 47000/mm³ and there were pain and swelling of his left leg. Doppler ultrasound examination showed left femoral vein thrombus formation. Thereafter enoxaparin therapy was started for deep vein thrombosis. Five days later when platelets were found to be 22000/mm³ the patient was consulted with one of our hematology team members. As the patient had no other explanation for thrombocytopenia HIT type II was strongly suspected. Anti-heparin platelet factor-4 (Diagnostica Stago, France) complexes were positive (OD 2,375). Both functional assays, heparin-induced platelet aggregation test (HIPA) and C14-serotonin release assay were positive (specific weight heparin therapy and unfractionated heparin during hemodialysis sessions were stopped. Fondaparinux 2.5 mg daily was started. We could not monitor the anti-Xa activity because of technical problems. During fondaparinux treatment platelet count increased to 193000/mm³ and repeated Doppler ultrasound showed recanalization of left femoral vein thrombus. When the platelet count reached 10000/mm³ oral anticoagulation with warfarin was initiated. The dose of warfarin was adjusted to maintain a target INR of 2.5. When INR was therapeutic for two consecutive days fondaparinux was stopped. During follow-up no new thromboembolic attack was observed. We present a patient with HIT type II and femoral vein thrombosis while on dialysis who was successfully treated with fondaparinux. In this case HIT type II and catheter-induced vessel wall damage were two independent risk factors for venous thrombosis. As HIT type II is a life threatening complication of heparin therapy all physicians using heparin anticoagulation should be aware of it. For all patients receiving unfractionated heparin alternate day platelet counts should be performed from days 4 to 14, as fondaparinux is too small to be recognized by the majority heparin-reactive antibodies it could be a reasonable alternative anticoagulant for symptomatic HIT type II patients where licensed drugs like lepirudin and danaparoid are not available.

**1433**

**INCREASED RISK OF DEVELOPMENT OF HEPARIN INDUCED THROMBOCYTOPENIA (HIT) IN ICU CRITICALLY ILL PATIENTS WITH VENOUS OR ARTERIAL LINES IN PLACE AND/OR NEED OF RENAL REPLACEMENT THERAPY WITH CONTINUOUS VENOUS-VENOUS HEMODIALYSIS FILTRATION (CVVHDF)**

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To investigate whether the use of heparin as anticoagulant in either central venous, arterial or CVVHDF catheters, as well as Low Molecular Weight Heparin (LMWH) may increase the risk of HIT. The main objective of the study is the follow-up and appropriate treatment of this critically ill population, given that early diagnosis of HIT reduces mortality from 30% to < 10%. Thirty (30) patients (17 men and 13 women) aged 31-87 years, admitted in the ICU for various reasons during 2004-2005 were included in the study. At admission, APACHE II score was <20 in 14 patients and > 20 in the remaining 16 patients. All patients had arterial or central venous catheters flushed with small quantities of unfractionated heparin. In 14 patients (group A) LMWH was administered in every day basis as prophylaxis from deep vein thrombosis (enoxaparine 2000-4000 IU/day), while 6 patients (group B) underwent CVVHDF using unfractionated heparin as anticoagulant. Platelet measurements were performed in all patients at day 1st, 7th and 15th with hematology analyzer ADVIA120. Antibodies against the complex heparin-PF4 with elisa (Ass erachrom EPIA, Stago) were tested in all patients at day 9 and 16 after catheter insertion. Catheters +LMWH Catheters + CVVHDF

<table>
<thead>
<tr>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=24</td>
<td>n=6</td>
</tr>
<tr>
<td>1st day</td>
<td>7th day</td>
</tr>
<tr>
<td>Thrombopenia</td>
<td>-</td>
</tr>
<tr>
<td>HIT-IgG</td>
<td>-</td>
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</table>

From all patients studied (80), at day 7, 12 patients from group A and 2 patients from group B were positive for HIT-IgG antibodies. In addition, at day 15, other 5 patients (4 from group A and 1 from group B) were positive for HIT-IgG detection. Overall, 17 patients (56.6%) developed HIT-IgG antibodies (seroconversion). Thrombocytopenia (HIT) was detected in patients (6,6%). All patients with HIT-IgG antibodies underwent vascular imaging (triplex) in order to exclude subclinical thrombosis. No correlation was found between severity score (APACHE II) and presence of HIT-IgG antibodies. According the results of this study, combination of heparinized catheters or use of LMWH seems to increase the incidence of HIT(6,6%) as well as the development of HIT-IgG antibody (seroconversion) in about 38,3% of patients. On the other hand, combined use of heparinized catheters and CVVHDF filters seems to increase the presence of HIT-IgG antibodies (seroconversion) in high percentage of patients (50%). The management of ICU patients with HIT includes:

- Discontinuation of LMWH
- flushing catheters with normal saline
- Use of filters without need of heparin.
1434
SEMINAL FACTOR VII AND FVIIA: THE ULTIMATE EVIDENCE ON THE PRESENCE OF THE TISSUE FACTOR DEPENDENT PATHWAY IN HUMAN SEMEN
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1Southampton University Hospital NHS Trust, SOUTHAMPTON, United Kingdom; 2University of Portsmouth, PORTSMOUTH, United Kingdom; 3American Diagnostica Inc, STAMFORD, CT, USA

Backgrounds. Human semen spontaneously coagulates into a semisolid mass and then wholly liquefies in a process that may have some similarity to that of blood. Besides other active components of the haemostatic system, semen contains a significant amount of functional tissue factor (TF). Aim: To investigate the presence of Factor (F) VII and FVIIa in human semen. Materials and Methods. Using a PT/APTT one stage factor assay and an Imubind™ FVIIa ELISA-assay, FVII and FVIIa levels were assessed in 97 semen specimens obtained from sub-fertile, normally fertile, fertile sperm donors and vasectomized subjects. Results. FVII and FVIIa were quantifiable in human semen. The mean FVII levels were 4.4 IU/dl and FVIIa were 12 ng/ml. Despite the observed variations in seminal FVIIa levels we found no significant differences in FVIIa levels among the studied groups. Seminal FVII levels showed a significant positive association with semen liquefaction time, sperm motility and semen volume. The anti-sperm antibodies and sperm-agglutination groups also showed raised FVIIa levels. We found no relationship between FVIIa levels and total sperm concentration (density), sperm counts per ml, sperm progression and days of abstinence. Conclusion. The present findings reinforce the concept of an active clotting system in human semen, not least the presence of the TF-dependent pathway.

1435
NO EFFECT B-VITAMIN SUPPLEMENTATION ON MARKERS OF INFLAMMATION IN PATIENTS WITH VENOUS THROMBOEMBOLISM
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Backgrounds. Mild hyperhomocysteinemia is associated with an increased risk of venous thromboembolism (VTE) and other cardiovascular diseases. Recent studies have suggested a role of inflammatory markers in the etiology of VTE and elevated homocysteine levels may contribute to low-grade inflammation. Vitamin supplementation with folic acid and B-vitamins was previously shown to decrease homocysteine levels. Aim: This study was designed in order to evaluate the correlation between homocysteine and markers of inflammation and to evaluate the effect of vitamin supplementation on these markers in patients with VTE. Methods. This study was a multicentre, randomized, double-blind, placebo-controlled trial. We randomized 105 patients with a first event of objectively confirmed VTE, aged between 18 and 70 years, to receive either vitamin supplementation (folic acid 5 mg, vitamin B6 50 mg and vitamin B12 0.4 mg) or placebo. Blood was collected at randomization and 8 weeks after the intervention period. Results. Ninety-nine (94.3%) completed the 8-week period of treatment. There was no difference in the levels of C-reactive protein (hsCRP) between patients with homocysteine above the highest tertile (12.6 µmol/L) and patients with below the lowest tertile (9.9 µmol/L). Median hsCRP was 0.28 mg/dl in patients with higher homocysteine levels and 0.19 mg/dl in patients with lower homocysteine levels (p=0.29). There was also no difference in the levels of interleukin-8 (52.5 and 59.5 pg/mL, respectively, p=0.51) between the two groups. In the patients treated with vitamins, there was a 29% decrease in the homocysteine levels. However, the levels of hsCRP and IL-8 did not change both in the vitamin and in the placebo-treated patients. Besides, treatment with vitamins had no effect on these markers even in patients above the highest tertile of homocysteine. Conclusion. In patients with VTE, higher homocysteine levels were not associated with increased levels of inflammation markers and homocysteine reduction by B-vitamin supplementation had no effect on these markers.

1436
PRIMARY HEMOPHAGOCYTIC SYNDROME CASE REPORT
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An 11 year-old white girl with good features presented with a history of fever, somnolence, jaundice and petechiae. She had a past history of jaundice and elevation of transaminases five months ago which resolved without therapy. There was hepatomegaly and splenomegaly 3 and 4 cm respectively. Laboratory findings were: Hb=8.4 g/dl, WBC: 1600/mm³, PLT:1800/mm³, ALT:290 U/L, AST:129 U/L, T bulirubin:12.5 µmol/L, D Bilirubin:4.5 mg/dL, PT:17.4 sec, PTT:42 sec, INR:1.5, fibrinogen: 90 mg/dl, serum ammonia: 150 mg/dl. Cerebrospinal fluid examination was normal. Clinical presentation was suggesting hemophagocytic lymphohistiocytosis (HLH) but bone marrow aspiration did not confirm the diagnosis. Marrow biopsy revealed hemophagocytosis. Hepatitis B, Hepatitis A, CMV, EBV, HSV, parvovirus serology did not show acute infection. Hepatitis A IgG was positive, HCV-RNA was negative. HLH-94 protocol was started and fever, hepatosplenomegaly and somnolence subsided but only partial hematological remission could be achieved (Hb:6.1 g/dl, WBC:4900/mm³, PLT:47000/mm³). ATG 10 mg/kg/day for three consecutive days were also administered and complete hematological and clinical remission was achieved. Parents were cousins but there was no history of similar disease. Genetic study could not be performed to confirm primary HLH. Following three uneventful years the patient was referred to our center again with a 5 month history of bilateral abducent paralysis and ataxia. Cranial MRI showed increased T2 signal in cerebellar, supratentorial areas, right occipital deep white matter, bilateral talamus and centrum semioval and diffuse cerebellar and mild cortical atrophy. HLH-94 protocol was again started but progression was seen with head tremor, generalized clonic convulsion, fever (59°C axillar) and cytopenias. Hb:10.6 g/dl, WBC:2150/mm³, ANC:678/mm³, PLT:79400/mm³, ALT:1586 U/L, AST:902 U/L, Fibrinogen 440 mg/dl, ferritin:160 mg/mL. Genetic study showed homozygotic perforin mutation that leads to aminocid acid exchange from (Val50Thr). We are planning allo-genetic stem cell transplantation from siblings if they do not show the same homozygotic perforin mutation because clinical presentation might be late as seen in the patient. HLH must be remembered in the differentiation diagnosis of fever, cytopenias, splenomegaly, hepatic failure and/or neurological symptoms. Delay in diagnosis may impair outcome. Allogenic stem cell transplantation is the only curative therapy in primary disease.

1437
HERPES GROUP VIRUS INFECTIONS AND GRANULOCYTOTOXIC ANTIBODIES IN CHILDREN WITH NEUTROPENIAS
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Relation between the duration of granulocytotoxic antibodies (GCTA) circulation and the presence of herpetic infection has been studied in children with immune neutropenias. Group 1 consisted of 15 herpes group virus-infected children with immune neutropenia aged 4 to 24 months; virus infections included cytomegalovirus (n = 10), Epstein-Barr virus (n = 1), herpes simplex virus 1 or 2 (n = 2) and mixed infection (n = 2). GCTA circulation lasted for 0.5 to 3 months (1.67 ± 0.25). GCTA titers ranged from 1:2 to 1:64. No correlation between GCTA titers and duration of GCTA circulation has been revealed. Group 2 consisted of 18 children aged 6 to 12 months with immune neutropenia and no markers of herpetic group viruses. GCTA circulation lasted for 0.5 to 3 months (2.3 ± 0.4). GCTA titers ranged from 1:4 to 1:156 and, similarly to those in Group 1, caused no effect on the duration of GCTA circulation. Thus, statistically significant difference in duration of GCTA circulation (p < 0.001) between the studied groups has been found, this result indicates the presence of a pathogenic role of herpes group viruses in the immune conflict in children with immune neutropenias.

1438
THE VALUE AND LIMITATIONS OF WBC DIFFERENTIAL FLAGS PROVIDED BY THE AUTOMATED HEMATOLOGY ANALYSER SYSMEX XT 2000i
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Fundeni Clinical Institute, BUCHAREST, Romania

Backgrounds. SYSMEX XT 2000i is a fully automated hematology...
analysed with a throughput of 80 samples per hour. The analyser can provide different indicators including morphology and complete white blood cell differential using flow cytometry by semiconductor laser enabling a sophisticated analysis based on RNA/DNA content, cell size and inner cell complexity. The analyser detects the presence of abnormal or immature WBC, providing a list of suspect messages generated from abnormal cell locations on WBC/DIFF scattergram. Aims: The present study evaluates the performance of Sysmex XT 2000i in detecting of abnormal or immature WBC in comparison with manual microscopy review and also the value of flags in leucocyte count. Methods: In this study were included 100 samples. All venous blood specimens were collected from 100 patients admitted in Hematology Department between July 2005 and February 2006 and diagnosed as acute leukemia (44 cases), CLL (15 cases), malignant lymphoma (11 cases), chronic myeloproliferative disease(9), MDS(9), anemia(5), trombocytopenia(5), infectious mononucleosis(2), HCL(1), ITP(1), MM(1).Clinical sensitivity and specificity of suspect flags of XT were assessed by comparison with microscopy differential counts. Results. Sysmex XT 2000i generated messages of blasts in 95% of cases confirmed in 61% with optic microscopy. In the group of 44 cases with acute leukemia, XT flagged blasts in 41 cases (93%) compared with optic microscopy which detects blasts in 43 cases (96%). In MDS cases (8), 5 samples(62%) were flagged on XT and optic microscopy confirmed presence of blasts in all 8 cases. 15 samples of CLL were false positive for blasts on XT, in all cases we found mature lymphoid cells. XT flagged blasts on XT need a manual microscopic review.Symsnex XT 2000i shows high sensitivity (95%) and lower specificity (50%) in detections of blasts, provide reliable results and a WBC differential comparable with optic microscopy.

1440

ORAL VORICONAZOLE AS SECONDARY PROPHYLAXIS DURING ALLOGENIC STEM CELL TRANSPLANTATION IN A PATIENT WITH PREVIOUS SEVERE INVASIVE FUSARIUS SP INFECTION


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Invasive fungal infections (IFI) represent one of the most challenging complications in patients submitted to stem cell transplantation (SCT). Invasive fusariosis has a high mortality rate in immunocompromised patients. Retrospective analysis of 69 brazilian patients (Nucci et al, CID 2004) showed a median survival of only 13 days. None of this patients, although received a newer antifungal drug, such as Voriconazole. Some studies have showed the safety and efficacy of Voriconazole as a secondary prophylaxis during SCT in patients with previous Aspergillus sp infection. However, to our knowledge, the use of Voriconazole as a secondary prophylaxis in SCT in patients with previous invasive Fusarius sp infection has not been reported yet. Case Report. A 49 years old man, successfully received a SCT in 2001, developed 4,5 years thereafter a secondary AML in the cells of the donor (Pironi et al. BMT in press). An attempt to induce a remission with standard treatment (Cytarabine and Daunorrubicin) failed. A second course was started. Routine chest evaluation during febrile neutropenia showed halo sign on computed tomography (TC) and deoxicolated Amphotenicin was started. After two days, with the appearance of skin lesions and important myalgia a clinical suspicion of fusariosis was made and oral Voriconazol was started. Fusarium sp blood culture sample confirmed the diagnosis of invasive fusariosis. He achieved complete remission after salvage chemotherapy and used oral Voriconazole for 2 months. The latest pulmonary CT scans showed a very small residual lesion in the lower lobe of the right lung. With two other sibling identical donors, we submitted him to a second SCT with a different donor. During the whole conditioning period (Bussulfan 16 mg/kg and Fludarabine 120 mg/m²) and until day +6 he received 400 mg of oral Voriconazole. Liver and renal test were undertaken daily; and Cyclosporine level were measured twice a week. A week increase in bilirubin on day +6 (6,48 mg/dL) Voriconazole was stopped for two days and reintroduced two days half hour the dose (200 mg). He had no further complications, except for a grade II mucosites. Bone marrow take was on day +18. Routine weekly CT chest scans don’t reveal any radiological signal of Fusarium sp infection reactivation.

Conclusions. At the best of our knowledge this is the first described case of successful secondary prophylaxis with Voriconazole in a patient with previous severe disseminated fusariosis submitted to a stem cell transplantation. Since fusarium infections have a trimodal distribution after SCT (Nucci et al. CID 2004) further cautious follow up will be necessary in this case. But we can conclude that oral Voriconazole seems to be an important new drug that can be safely used for secondary prophylaxis during SCT in patients with previous invasive Fusarium sp infections.

1441

EFFICACY OF PAROMOMYCIN AND / OR AZITHROMYCIN IN HEMATOLOGICAL PATIENTS WITH CRYPTOSPORIDIOIS

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Cryptosporidium parvum is a widespread parasite of the Apicomplexa genre, with a fecal-oral way of transmission and capable of overcoming chlorination of water. Intestinal cryptosporidiosis as a cause of severe diarrhea is not uncommon in underdeveloped countries. Since the 80’s, a rising incidence occurred in Western countries, related to HIV pandemic. There are only isolated cases of the infection in the setting of hematological disease. Diagnosis requires a high level of suspicion and several explorations, often unsuccessful. There is no consensus on therapy, although a number of treatment options seem rather unsatisfactory. Nitazoxanide or long-term use of Paromomycin, Espiramycin and/or Azithromycin p.o. have been proposed, without certainty of eradication. Case t: 66 yr.-old male. Diagnosed of myelodysplastic syndrome, AREB5q-, with multiple
infectious episodes. In March-02, he was admitted to Hospital with fever, loss of weight (12 kg), diarrhea with >10 depositions/day, of liquid orange stools devoid of blood, tenesmus and abdominal tenderness with peritoneal signs. X-ray of abdomen showed diffuse dilatation of gut. Abdominal ultrasound scan and TAC revealed scarce free liquid and thickening of colonic wall. Colonoscopy: replacement of normal mucosa by multiple nodules, resembling sessile polips; a biopsy of one of them was informed of 'minimal inflammatory changes'. Conventional microbiologic studies rendered no result (cultures, C. difficile toxin, serologies, search for virus and parasites). Specific search for C. parvum (modified Ziehl’s stain) was positive in 3 samples. The CD4+ lymphocyte count was 400/µL. Therapy: paromomycin, 1 g p.o. b.i.d. and diet supplementation with Lacto-bacillus sp. Resolution of diarrhea, a significant weight gain and improvement in performance status was attained in the following weeks. Case 2: 18 yr.-old male. Diagnosed of Hodgkin’s disease, NS-subtype, stage IV-B, refractory to several lines of chemotherapy, including BEACOPP, ESHAP/MINE and a gemcitabine ‘based scheme. In April-05, he was admitted to Hospital because of protracted fever, diarrhea with green liquid stools, loss of 4 kg in a single week, diffuse abdominal pain, vomiting and tenesmus. Conventional microbiologic studies were also inconclusive. Considering the former case, we asked again for a C. parvum search in stools, which was clearly positive. Therapy with azithromycin (5 days) and paromomycin (14 days) was undertaken, after which diarrhea and fever, as well as the other symptoms disappeared in this period; complete clearance of parasite cysts in stools could be demonstrated. A significant recovery of nutritional status was also accomplished. 1.- Conventional methods for detecting parasites in stools may not detect C. parvum. This protozoan must be suspected when no diagnosis can be drawn after a complete set of explorations, and an intentional search with specific stains. Although nitazoxanide has been approved for Cryptosporidiosis, this drug is not available in Spain yet. We believe that this simple combination, i.e., azithromycin, paromomycin and diet supplementation is a suitable option for an, otherwise emaciating, unusual form of infectious diarrhea in hematological patients.

**1442**
ATYPICAL EOSINOPHIL DISTRIBUTION OBSERVED IN PATIENTS WITH MALARIAL INFECTION WHEN USING SYSMEX XE-2100

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**Backgrounds.** The incidence of malaria has been increasing in civilian population and the prevalent area being wider in Korea. Malaria must be recognized promptly in order to treat the patient in time and to prevent further spread of infection in the community. **Aims.** Malaria can be suspected based on the patient’s symptoms and the physical findings at examination. However, for a definitive diagnosis to be made, laboratory tests must demonstrate the malaria parasites or their components, which are time consuming and need expertise. As it is likely that automated screening tests like a complete blood cell count are always undertaken for patients who present with pyrexia, it can be expected that attention to any abnormalities found in automated hematology analyzer can decrease a delay in the diagnosis of malaria if such a diagnosis was not initially considered.

**Methods.** Hematological analysis using Sysmex XE-2100 (TOA medical Electronics, Kobe, Japan) and Advia 120 (Bayer Diagnostics, Tarrytown, NY, USA) was performed on samples positive for malarial parasite. **Results.** We found 3 peculiar patients with P. vivax malaria who had pseudoeosinophilia determined only when using Sysmex XE-2100. Although eosinophilia of 5.4%-24.3% was found in 3 patients when measured by Advia XE-2100, eosinophilia was not found either when measured by Advia 120 or read by microscopy. As a result of reviewing the scattergram generated by Sysmex XE-2100, atypical eosinophil distribution was placed more closely to the neutrophil distribution than typical eosinophil distribution in the WBCs scattergram (Fig. 1). This atypical eosinophil distribution was due to the presence of hemozoin-containing neutrophils. It was concluded Sysmex XE-2100 analyzer showed abnormal eosinophil counts. Since it is feasible that reading the WBCs scattergram to find a certain hematologic abnormality such as atypical eosinophil distribution as a result of hemozoin-containing neutrophils may contribute to the diagnosis of malaria especially for patients unsuspected.
diagnosed with mantle cell lymphoma because of splenomegaly and hypoalbuminemia. The patient had stage IV with bone marrow involvement. The patient was treated with a combination of rituximab (375 mg/m² D1) and chemotherapy with standard CHOP: cyclophosphamide 750 mg/m² D1, doxorubicin 50 mg/m² D1, vincristin 2 mg D1, and oral prednisin 100 mg D1 to D5 given in 3-week cycles. She received eight cycles of treatment. Evaluation after the eight cycles showed a complete response of 5 weeks. One month after the last chemotherapy, the patient presents rapidly psychiatric disturbances with speech dysfunction and paranoia delirium. PML was suspected after magnetic resonance imaging with frontal and temporal leucocencephalopathy. JC viral DNA was detected in the cerebrospinal fluid. HIV serology was negative. Low level of CD4, CD8, B lymphocytes and NK cells were noted. A treatment with cidofovir was started. Two months after the beginning of symptoms, neurological disturbances were stable.

Results. A few cases of PML were recently described in patients who were treated with chemotherapy, transplantation and peritransplantation rituximab. A direct association between rituximab and PML remains speculative. Moreover, the patients reported were often in relapse, heavily pre treated. Our patient is, to our knowledge, the first case of PML after a combination of CHOP with rituximab, in first induction procedure. Conclusions. Unusual viral infections were recently described in patients treated with high dose chemotherapy and rituximab. Although the contributory role of rituximab remains speculative, our additional case highlights the need for an accurate surveillance, even in patients not heavily pre treated, in first induction with CHOP and rituximab.

**1445**

**VARIEABLE RESPONSE TO CURRENT TREATMENT OPTIONS IN HYPEREOSINOPHILIC SYNDROME (HES): A SINGLE CENTRE EXPERIENCE**

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**Backgrounds.** Hypereosinophilic syndromes (HES) refers to a heterogeneous group of disorders characterised by marked blood eosinophilia (>1500/cu mm) and tissue eosinophilia (lasting for more than 6 months), in the absence of other etiologies for eosinophilia, resulting in end organ damage. HES may be a reactive condition or a chronic myeloproliferative disorder (with evidence of clonal proliferation). Reactive eosinophilias are due to release of cytokines (IL-3, IL-5, GM-CSF etc) and the common causes are parasitic (helminthic) infections, allergic diseases, vasculitides drug reactions and malignancies. Clonal eosinophilias are those in which the eosinophilia is a part of a clonal haematological malignancy, which is very often associated with the fusion gene FIP1L1-PDGFRα, which causes the generation of a constitutively active Tyrosine Kinase. Several visceral complications like cardiomyopathies, nervous system involvement (e.g. paraparesis, cerebrbral infarction, eosinophilic meningitis etc) are often fatal illnesses. Treatment modalities for HES includes corticosteroids, chemotherapeutic agents (hydroxyurea, cyclophosphamide vincristine) and interferon. Newer treatment modalities including tyrosine kinase inhibitors (e.g. Imatinib) and monoclonal anti-IL5 antibodies are now available. Patients carrying this fusion gene respond well to the Tyrosine Kinase Inhibitor Imatinib. Some patients with HES, that are negative for this fusion gene may also respond to Imatinib, suggesting that in such cases other Tyrosine Kinases may be dysregulated.

**Methods.** The 5 patients (4 Male, Female; age range 37-80 yrs; mean age 56 yrs) presented with eosinophilia in the range 2600-12,000/cumm. A treatment to was performed as Eosinophil count < 1500 (cumm) or Eosinophil count < 5% of the total leucocyte count in the peripheral blood. 5 of the 5 patients received Imatinib as initial treatment. 1 patient initially had Methyl Prednisolone followed by Imatinib and 1 patient (aged 80 yrs) was treated with Hydroxyurea initially. Results. Two of the four patients receiving Imatinib responded to it. Of the 2 patients not responding to Imatinib, 1 responded partially to Hydroxyurea and the other did not respond to monotherapy with steroids or α-interferon. However the latter eventually responded to a combination of steroids and α-interferon. The patient who had initial treatment with hydroxyurea responded well. Of the 5 patients 1 was equivocal (possibly false positive) for the FIP1L1-PDGFRα fusion gene. Two were negative and two were not tested. Of the 2 that were negative 1 responded to Imatinib (see Table). Summary. Thus response of HES patients to the various treatment modalities is variable and often unpredictable. A trial of Imatinib is worth considering in all cases. In that case that are refractory to monotherapy with Imatinib, steroids and α-interferon, a combination of the last two agents may be tried. In difficult resistant cases of HES, monoclonal anti II. 5 may be tried.

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<th>Table 1. Hypereosinophilic syndrome.</th>
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<td>Patients</td>
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M: Male; F: Female.

**1446**

**SIBLING MATCHED HSCT CAN RESTORE HEMATOPOIESIS AND STROMAL TISSUE IN PATIENTS WITH IDIOPATHIC MYELOFIBROSIS**

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Natural history of idiopathic myelofibrosis (IMF) is hardly modified by conventional therapy. Neither hydroxycarbamid nor IFNα and busulfan can restore normal hematopoietic function. Fibrosis of the marrow constitutes a characteristic feature of the disease. The parenchymal tissue lesions could make decision of hematopoietic stem cell transplantation (HSCT) more difficult, as it is not known, whether normal function of marrow supported tissue could be restored after transplantation in advanced cases. Since 2000 year five patients with IMF received sibling matched HSTC (R/M 4/1, age 29 - 55 yrs, median age 46, all patients were in 3 stage of the disease according to the WHO pathological staging). All patients fulfilled 4 diagnostic criteria established by PVSG and were in high or intermediate risk group of the disease according to the Dupriez prognostic scoring system. Two of them received myeloablative conditioning: BuCy2, three nonmyeloablative conditioning: Bu or Me, Flu and ATG prior to PBPC transplantation. One patient died 30 days post transplantation in the course of EBV reactivation (7345 viral copies/100,000 cells) with allergic vasculitis et hemolysis. Four patients are alive and well from 3 to 70 months post HSCT.

Hematological recovery was prompt and followed by resolving of fibrosis easily seen as soon as 30 days after transplant. Normal marrow trephine biopsy pictures were found by six month post transplant. However, proportion of mesenchymal stem cells (CD45-, CD34-, CD105-, CD 73+, CD 90+) was lower in the marrow of IMF patients as compared to CML cases at the same time post transplant. All patients including fatal one were full chimeras by one month post HSCT. In conclusion, normal hematopoietic and stromal tissue can be restored by HSCT in IMF cases at advanced stage.

**1447**

**DETECTION OF JAK2V617F MUTATION IN MYELOPROLIFERATIVE DISORDERS: CLINICAL CORRELATIONS.**

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Ph-negative myeloproliferative disorders (MPD) include three major disorders presented by polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (IM). All reflect clonal transformation of a multipotent hemopoietic progenitor. Overlapping clinical features and undefined pathogenesis are responsible for diagnostic and prognostic problems. Recently, several groups have identified a frequent single point mutation (V617F) in the JAK2 gene (JAK2V617F), sug-
Fusion gene has been identified in 50% of cases of HES. This came back as negative but he was thus Imatinib is very useful therapeutic. We retrospectively assessed the diagnostic criteria from other causes of erythrocytosis. Recently it has been shown and the World Health Organisation (WHO) to diagnose PV and differentiate it from other causes of erythrocytosis. Criteria have been established by the Polycythaemia Vera Study Group (PVSG), the British Society for Haematology (BSH) in which the dominant feature is excessive erythropoiesis resulting in a raised red cell mass. Criteria have been established by the Polycythaemia Vera Study Group (PVSG), the British Society for Haematology (BSH) and the World Health Organisation (WHO) to diagnose PV and differentiate it from other causes of erythrocytosis. Recently it has been shown that PV is associated with an acquired activating mutation, V617F, of the Janus kinase (JAK2). Aims. We retrospectively assessed the diagnostic information of patients with erythrocytosis of all causes (PV, idiopathic, secondary and apparent) against the BSH criteria. We determined if a diagnosis of PV would have been established by these criteria and whether or not this agreed with the diagnosis made by their clinician. We are determining the JAK2 status of this group. Methods and Results. The patient sample was drawn from a clinical database. The records of 77 patients who attend Belfast City Hospital with PV (47 patients) and other causes of erythrocytosis (30 patients) were reviewed and relevant information was recorded. Sufficient data was available to apply the BSH criteria to 64 PV, 36 other PV (83%). Thirty-five patients met the BSH criteria for a diagnosis of PV and 29 patients did not. There was agreement with the diagnosis established by the patients clinician in 65 out of 66 cases. Only 1 patient had been diagnosed with PV who did not meet the BSH criteria. This patient met both the WHO and PVSG criteria for PV. To date the JAK2 V617F mutation has been demonstrated in 29 out of 31 tested patients with PV and in 1 of 12 patients with erythrocytosis of other causes. Conclusions. We concluded that the BSH criteria for the diagnosis of PV were easily applied, inclusive and specific. Results of V617F JAK2 mutation analysis are consistent with previous findings and support the suggestion that this should be incorporated into the initial evaluation of patients with erythrocytosis.

Case History. We present a 45 year old man, with background history of allergic rhinitis and asthma, who presented with left sided weakness and slurred speech. A MRI scan showed a right lacunar infarct. A CT scan done after 4 days revealed a large right intra cranial haemorrhage. He also had transoecal echocardiogram (TOE) evidence of mitral valve endocarditis which was treated with appropriate antibiotics. His FBC revealed a eosinophil count of 12 Further investigations revealed no evidence of a parasitic infection or drug allergy. As an inflammatory aetiology was initially suspected he was initially commenced on Methyl Prednisolone which was later changed to oral Prednisolone (1 mg/kg). A subsequent Brain Biopsy was negative for vasculitis and bone marrow aspirate/biopsy revealed increased eosinophil precursors but there was no evidence of lymphoma. Molecular studies of marrow aspirate revealed no evidence of clonal T cell rearrangements. There were no metaphases noted on cytogenetic analysis of the aspirate. The aspirate was sent away for detection of the FIP1L1-PDGFRA by RT-PCR. His eosinophil count fell to 3.5-4 on oral Prednisolone. A trial of Imatinib was considered appropriate. This was commenced at a dose of 400mg/day. Results. A response in the eosinophil count (=0.84) was noted in 3 days. Over the next 2-4 weeks the eosinophil count fluctuated but remained between 0.8 and 2.0. The Prednisolone was gradually tapered off to zero. FIP1L1-PDGFRA came back negative. The Imatinib dose was reduced to 200 mg per day after 3 weeks. When he was discharged, after a month later his eosinophil level was 0.78 and his maintenance dose of Imatinib was 100 mg/day. Discussion: It’s worth noting from this case that a trial of imatinib is worthwhile after all the necessary blood tests have been sent. We note that this patient’s eosinophilia responded to steroids but the count came down to near normal levels after 4 weeks of Imatinib. The result of his FIP1L1-PDGFRA came back negative but he was clearly an Imatinib responsive HES. Thus he may have one of the other very rare fusion genes associated with eosinophilia (causing dysregulation of the enzyme Tyrosine Kinase), which respond to Imatinib but are difficult to test in the absence of karyotypic abnormalities.

**1449** RESPONSE TO IMATINIB IN A FIP1L1-PDGFRA α NEGATIVE HYPEREOSINOPHILIC SYNDROME (HES) PATIENT WITH STROKE AND INFECTIVE ENDOCARDITIS

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**Backgrounds.** The Hypereosinophilic syndromes are a rare haematologic disorder characterized by eosinophilia (>5.0 × 10^9/L) persisting for more than 6 months in the absence of reactive causes. Recently the FIP1L1-PDGFRA fusion gene has been identified in 50% of cases of HES. This fusion gene is a constitutively activated tyrosine-kinase. Imatinib mesylate, which is a selective inhibitor of several Tyrosine Kinases, including PDGF receptors. FIP1L1-PDGFRA fusion gene seems to be another target of this drug.

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**1448** EVALUATION OF THE BRITISH SOCIETY FOR HAEMATOLOGY CRITERIA FOR THE DIAGNOSIS OF POLYCYTHEMIA VERA

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**Backgrounds.** Polycythaemia vera (PV) is a myeloproliferative disorder in which the dominant feature is excessive erythropoiesis resulting in a raised red cell mass. Criteria have been established by the Polycythaemia Vera Study Group (PVSG), the British Society for Haematology (BSH) and the World Health Organisation (WHO) to diagnose PV and differentiate it from other causes of erythrocytosis. Recently it has been shown that PV is associated with an acquired activating mutation, V617F, of the Janus kinase (JAK2). Aims. We retrospectively assessed the diagnostic information of patients with erythrocytosis of all causes (PV, idiopathic, secondary and apparent) against the BSH criteria. We determined if a diagnosis of PV would have been established by these criteria and whether or not this agreed with the diagnosis made by their clinician. We are determining the JAK2 status of this group. Methods and Results. The patient sample was drawn from a clinical database. The records of 77 patients who attend Belfast City Hospital with PV (47 patients) and other causes of erythrocytosis (30 patients) were reviewed and relevant information was recorded. Sufficient data was available to apply the BSH criteria to 64 PV, 36 other PV (83%). Thirty-five patients met the BSH criteria for a diagnosis of PV and 29 patients did not. There was agreement with the diagnosis established by the patients clinician in 65 out of 66 cases. Only 1 patient had been diagnosed with PV who did not meet the BSH criteria. This patient met both the WHO and PVSG criteria for PV. To date the JAK2 V617F mutation has been demonstrated in 29 out of 31 tested patients with PV and in 1 of 12 patients with erythrocytosis of other causes. Conclusions. We concluded that the BSH criteria for the diagnosis of PV were easily applied, inclusive and specific. Results of V617F JAK2 mutation analysis are consistent with previous findings and support the suggestion that this should be incorporated into the initial evaluation of patients with erythrocytosis.
1450
THROMBOCYTOSIS. ETIOLOGIC ANALYSIS OF 1688 HOSPITAL PATIENTS
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Background-Aims-Methods: One thousand six hundred eighty eight hospital patients aged 21 to 86 years with thrombocytosis (defined as a platelet count of more than 450,000/cu mm and below 1,000,000/cu mm in 97% of patients) seen in our hospital over a 15-year period, were studied prospectively for etiological diagnosis. Results: The causes of thrombocytosis were myeloproliferative diseases (6%), malignancy (17%), post-surgery or experiencing tissue damage massive acute hemorrhage or thrombotic episodes (19%), infections (34%), chronic inflammation (5%), iron deficiency anemia (10%), miscellaneous disease states as cardiac disease, liver cirrhosis, renal failure etc (9%). Thrombocytosis associated with multiple, simultaneous causative factors was seen in 7.9% of cases. Among all hospital patients with infections, sepsis was associated with a much higher platelet counts than any other infection (P < .0001). Thrombocytosis secondary to infections and malignancy was significantly more common in aged patients. No thrombocytosis-related complications were seen in any hospital patient and none required any specific treatment. Conclusions: Thrombocytosis is a frequent finding in hospital patients. It is due to a variety of etiologic factors and is of significance clinical discriminatory value. It is often due to an acute-phase phenomenon in response to infection, tissue damage, blood loss, or anemia, and is an early sign especially of disseminated, advanced or inoperable malignancy.

1451
PERICARDIAL EFFUSION IN MYELOPROLIFERATIVE DISORDERS
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Backgrounds: Extramedullary haematopoiesis is very common in Chronic Myeloproliferative Disorders (MPDs) and the commonest site of extramedullary haematopoiesis is the spleen and the liver. Unusual sites can sometimes be affected leading to haematopoietic tumours surrounded by a capsule of connective tissue. Such sites includes lymph nodes, CNS, skin, pericardium, peritoneum, pleura ovaries, GIT and the lung. Many such cases remain asymptomatic and may be diagnosed incidentally. However in cases where the pericardium is affected cardiac tamponade may result, requiring urgent intervention (pericardiocentesis). Recurrence may be prevented by minor pericardectomy. In patients with myeloproliferative disorders and increased cardiac silhouette on X-Ray film, with or without clinical heart failure an echocardiogram is recommended in order to identify a possible pericardial effusion. Treatment with radiotherapy have been shown to be effective. Case History: We report a 68 year old man who recently presented with weight loss, mild anaemia moderate thrombocytosis and leucocytosis. A diagnosis of MPD (unclassifiable) was made 3 years back but he had been transfusion independent. He has background history of atrial Fibrillation on Warfarin (thus his thrombocytopaenia might result in bleeding while he was on Warfarin). On examination he had 4 cm splenomegaly and 5 cm hepatomegaly. A bone marrow aspirate showed myeloid hyperplasia with no evidence of transformation. Cytogenetics revealed loss of α chromosome (of no significance). FISH for bcr/abl was negative. The trephine biopsy was consistent with a Myeloproliferative Disorder. Thus the diagnosis was MPD (unclassifiable). Five days after admission he developed diarrhoea which was treated empirically with oral Metonidazole after stool samples were sent.

As he was dehydrated, he was commenced on IV fluids and a further 48hrs later he developed bilateral pedal oedema up to his knees. He had clinical evidence of CCF. An ECG showed low voltage complexes and an Echocardiogram revealed a concentric pericardial effusion of 2.46 cm. The right atrium was not collapsing on inspiration. He was transferred urgently to the Coronary Care Unit (CCU) so that urgent pericardiocentesis may be done if he developed cardiac tamponade. He remained stable in the CCU. Serial echocardiograms did not reveal any evidence of increase of the effusion. A CT (thorax/abdomen) showed moderate pleural effusion, pericardial effusion with massive hepatosplenomegaly and mild ascites. At present he has required no intervention for his pericardial effusion. Radiotherapy may be a non surgical option for him in the future. Discussion: There are about 70 case reports of Pericardial effusion (in Myeloproliferative Disorder) in the literature (MEDLINE search), 7 of these are in Idiopathic Myelofibrosis, 1 in Essential Thrombocytosis, Reported in Chronic Myeloid leukemia and 1 in a case of MFD (unclassifiable). Conclusions: Myeloproliferative Disorder patients can develop moderate to severe pericardial effusion which can result in dramatic clinical deterioration; hence an high index of suspicion and an early echocardiogram necessary. Close monitoring in CCU will ensure that urgent intervention (pericardiocentesis) can be undertaken if cardiac tamponade develops.

1452
THE INCIDENCE OF THROMBOTIC EVENTS IN CHRONIC MYELOPROLIFERATIVE DISORDERS
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The thrombophilia due to Chronic Myeloproliferative disorders (CMD) is determine by modification of rheologic parameters through hyperviscosity, the perturbation on thrombocyte function and the perturbation of the cytokines secretion. The thrombophilic events associated to CMD are the result of the thrombophilia feature with the quality of medical care. This is the reason for the analysis of the 136 subjects diagnosed in our Clinics with CMD between 2000 - 2005. We diagnosed in 52 subjects Polycythemia Vera (PV) (32,3%), in 52 subjects Essential Thrombocythemia (ET) (23,3%), in 26 subjects Agnogenic Myeloid Metaplasia (AMM) (19,1%) and in 26 subjects Chronic Myeloid Leukemia (CML) (19,1%). There were diagnosed thrombotic events in 49,26% subjects: recurrent cerebral thrombosis in 28 subjects (20,58%): 34,46% in PV, 18,75% in ET, 7,79% in CML. Recurrent thrombophlebitis in 8 subjects (5,88%); 12,5% in ET, 7,69% in AMM and 3,84% in PV. Superficial thrombophlebitis: 2 subject in ET (6,25%); central retinal vein thrombosis: 2 subject in CML (7,69%); Disseminated intravascular coagulation: 2 subject in ET (6,25%); Splen infarction: 2 subject in CML (7,69%); Portal vein thrombosis: 4 subject (2,94%): 7,69% in AMM and 6,25% in ET; Arterially and capillary thrombosis 8 subject (5,88%); 11,53% in PV, and 6,25% in ET; Necrotizing purpura: 1 subject in TE (3,125%); Heart infarction: 9 subjects (6,61%): 15,625% in ET, 7,77% in PV; Mesenteric infarction 1 subject in ET (3,125%). The frequency of thrombotic events was 75% in ET, 61,5% in PV, 23,07% in CML and 15,82% in AMM. Conclusion: The thrombotic events are an important risk factor in CMD patients, even in early stages. Thrombotic events developed before the diagnostic of CMD and 25% after this. This impose to facility the access to modern therapy: Erythropoiesis, Anagrelide, Glivec (imatinib), α interpheron and to consider the primary and secondary thrombophilia as major risk factor for cardiovascular disease.

1453
WHY WE HAVE NO WORKING SYSTEM OF ELECTRON CASE HISTORY UNTIL NOW? EFFORTS TO DEVELOP THE NATIONAL STANDARD
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The computerized case history system allows the collection of medical information from multiple sources, integrated presentation, fast search, simultaneous availability to several participant of medical process etc. It is efficient for patient follow-up, consultation, and transfer as well as for analysis of clinical trials. The subsidiary role of information systems in medical process partly the result of insufficient attention is given to the question of the official status of electronic personal medical records and documents. In the majority of hospitals such systems are used only for preparation and printing of medical documents, which signed by ink, participate in traditional medical document circulation. Case history or research forms of clinical trials shelved in storage and if could to be retrospectively analyzed, only with huge efforts. It seems, that most of pharmacological companies, conducting clinical trials are...
not interested in direct collecting data in electron database, because such system able to provide real audit of study procedures and conclusions, practically impossible with paper form collection. Use of the electronic medical data and electronic archives demands to provide - an invariance and reliability during all period of storage; a regulation of rights of access and confidentiality; personification (an opportunity to define the author and an origin of record at any moment - analogue of the signature on the traditional document). Concerning the traditional medical documentation a lot of normative were developed. Electronic personal medical records needs the development of standards providing their legal status and an effective utilization in medicine and public health services. It’s so happened in Russia, that the project 'The National Standard of electronic case history' were presented by the National Center for Hematology.

1454
ERDHEIM-CHESTER DISEASE: TREATMENT WITH INTERFERON-α
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Erdheim-Chester (EC) is a rare non-Langerhans’ cell histiocytosis of unknown etiology. This rare illness has a particular tropism for connective and adipose tissues. There are typical radiographic and pathological features, which can lead to the diagnosis, but the clinical spectrum ranges from a focal asymptomatic process to a multisystemic, rapidly fatal, infiltrative disease. The entity is defined by a mononuclear infiltrate consisting of lipid laden foamy cells (greater than stain positively for CD68), and negativity for CD1a and S100. The differential diagnosis includes Langerhans’ cell histiocytosis, metabolic disorders, and malignancies. The outcome of patients is worse than that for Langerhans’ cell histiocytosis with about 60% of patients dead after a mean follow-up of 32 months, whereas only 9% of patients with the latter disorder have succumbed after a median follow-up of 4 years. Corticosteroids, chemotherapy, surgical resection, and radiotherapy have been used to combat EC disease and there is no consensus concerning the best treatment. A recent study showed good outcome of 3 patients with advanced disease but without pulmonary and cardiac involvement treated with interferon-α (IFN-α). The aim of this study is to present an advanced EC case with cardiac, pulmonary, bone, retro-orbital and retroperitoneal fibrosis that showed bad outcome of the cardiac function with IFN-α therapy. We evaluated a 48-year-old woman with a 2-year history of striking exophthalmos. Her previous history included retroperitoneal fibrosis with unknown cause and obstructive renal impairment that led to chronic kidney failure. The ophthalmologic examination revealed a Hertel ophthalmometry measurement of 27 mm (normal 12-20 mm). Computed tomography of the orbits showed massive infiltration. Retro-orbital mass biopsy was consistent with EC. Prior to the treatment the exams showed 46% of heart ejection fraction on the left ventricle, lung function with a mild restrictive ventilation defect and a simetrical involvement of long bones (sclerotic bone lesions in femora, tibiae and radii). IFN-α, was started at a dose of 3 x 106 units, subcutaneous, 3 times per week. After one month, the ophthalmometry measurement revealed a reduction of 2 mm in the right eye and 1 mm in the left eye. Two months later, the computed tomography of the orbits showed regression of 3 mm in retro-orbital mass bilaterally. Nevertheless, heart ejection fraction was reduced to 26% after the same period of treatment. Although there was an evident improvement of exophthalmos, the patient showed a pronounced decrease of the cardiac function. IFN-α has been widely used in clinical practice as an antiviral, anticancer, and immunomodulatory agent. On the other hand, IFN-α is known to induce adverse effects such as cardiac dysfunction, cardiomyopathy, various kinds of arrhythmias, and sudden cardiac death, although clinical trials which evoke these cardiac events are not documented. This aggravation could be, in part, imputed to the interferon-α therapy. So, the decision of use this therapy in patients with previous cardiac disfunction should be done carefully and a closely heart evaluation can be useful.

1455
COMPARISON OF TWO DIFFERENT TOP AND BOTTOM DEVICES FOR CORD BLOOD VOLUME REDUCTION
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The major problem with long-term cord blood banking is the required storage space. Red blood cell (RBC) depletion of cord blood (CB) collections not only maximises storage space but have also another advantages like the reduction of the amount of DMSO as cryoprotectant thus reducing the potential side-effects of DMSO and the reduction of side-effects of ABO mismatched or hemolyzed red blood cells. Our cord blood bank performs volume reduction with top and bottom devices. Aims. To compare two different top and bottom devices for the cord blood volume reduction purpose. The obstetrical team evaluated maternal and neonatal pairs during the pre-partum period in the maternity wards collaborating in the cord blood program. Donors signed informed consent before delivery, during last months of pregnancy. The CB units collected in triple bag system were centrifuged in open buckets at 3000 g for 2 min at 2°C, ensuring that the bags were well supported to prevent disruption of the buffy coat layer. CB collections were separated into plasma, red blood cells concentrate (RBCC) and buffy coat (BC) containing haematopoietic progenitors with two different devices: Optipress II and Compomat G4. A standard protocol programmed into the Optipress II, together with the standard backplate for BC preparation was used to process the CB units (n=27). The programme was set with the following parameters: BC volume of 40 ml, a BC level of 5.5 and a force of 25. Program CB1 in Compomat G4 device was empirically developed to reach a BC volume of 41 ml (n=31). Monitoring the TNC, RBC, CD34+ cells and CFU content in both pre-process and post-process CB units assessed the volume reduction process during the development phase of the study. Results. Table 1 shows the results of the development phase of the study. When the two devices were introduced into routine, lymphocytes recovery (79.6±10.9% for Optipress II and 77.5±5.5% for Compomat G4, p<0.001) and red blood cell depletion (58.6±1.6% for Optipress II and 51.4±1.9% for Compomat G4, p<0.005) were significantly better for CB units processed with Optipress II. TNC recovery was similar for both methods (78.5±7.8% for Optipress II and 78.2±7.2% for Compomat G4, p=ns). Conclusions. Compared to Optipress II, volume reduction with Compomat G4 device allows worse lymphocyte recovery and RBC depletion of cord blood units.

Table 1.

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<th>Optipress II</th>
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<td>CD34 recovery (%)</td>
<td>116.6±81.9</td>
<td>85.0±13.6</td>
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<td>CFU recovery (%)</td>
<td>93.6±26.8</td>
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1456
A VERY SMALL POPULATION OF CELLS EXPRESSING THE CD133 HEMATOPOIETIC STEM CELL ANTIGEN EXISTS IN HUMAN ADULT SUBSTANEA NIGRA AND STRIATUM BRAIN TISSUES
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Numerous animal studies have demonstrated the presence of neural stem cells in the mammalian forebrain. Recently, the CD133 haematopoietic stem cell antigen has been identified in foetal human brain tissue, human focal cortical dysplasia, premature infants cortex and in paediatric brain tumours. To date, it is unclear whether these stem cells exist in human adult brain tissues. The aim of the present study was to evaluate the presence of CD133 positive cells in the various areas of postmortem human midbrain and hindbrain tissues including the substantia nigra, stratum, medulla and pons. The immunocytochemical staining with an anti-CD133 epitope 1 clone (Miltenyi Biotech Ltd.) and anti-CD133 epitope 2 clone (29NC3, Miltenyi Biotech Ltd.) revealed the presence of CD133 epitope-2 and not epitope-1 in only two sites: substantia nigra and stratum in post-mortem brain tissue sections from 4 elderly patients purchased from Medical Solution plc. (Nottingham, UK). The CD133 epitope-2 positive cells were oval prolonged in shape and have size of 150-172 μm². The CD133 positive cells in the substantia nigra were larger than those in the Striatum. The lack of any expression of CD133 epitope-1 in adult brain is in line with previous PCR-studies. Also, we investigated the presence of any expression of the OCT-4 embryonic antigen in the same adult brain tissue sections. Only a small population of cells expressing OCT-4 was found only in the same two sites: Substantia Nigra and Striatum as shown in the figures. Since in the substantia nigra of the midbrain, degeneration of dopaminergic neurones is responsible for the debilitating motor dysfunction in patients with Parkinson’s disease. Further studies are warrant-
ed to explore the therapeutic role of these unique CD133 positive cells residing in the substantia nigra and striatum of the adult human brain.

1457
INDUCTION OF HLA-DR AND CD33 EXPRESSION ON GRANULOCYTES AFTER GM-CSF AND G-CSF ADMINISTRATION

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Background. The effect of granulocyte-macrophage colony stimulating factor (GM-CSF) and granulocyte colony stimulating factor (G-CSF) on granulocytes function and phenotype is complicated and depends on the dose, the route and the way (bolus, continuous) of growth factor administration. There are also differences between in vitro and in vivo effects. Aim: The aim of this study was to evaluate the effect of GM-CSF and G-CSF on granulocyte phenotypic expression. Methods. We determined the phenotypic expression of granulocytes obtained from peripheral blood of 29 patients with hematological malignancies (7 patients had AML, 7 ALL, 4 CML, 5 non-Hodgkin’s lymphomas, 2 myeloma, 1 aplastic anemia, and 3 MDS) and 7 patients with solid tumors. Blood samples were collected on the 1st and 2nd day of growth factor administration, as well as on days 5-20 after the final day of growth factor injection. Phenotypic analysis was performed by the alkaline phosphatase (APAAP) immunocytochemical technique, using a wide panel of monoclonal antibodies. In addition, the in vitro effect of GM-CSF in short term cell cultures was evaluated in 2 cases with AML, 2 with solid tumors and in 2 healthy individuals. Peripheral blood cells were suspended in RPMI with and without FBS 10%, with and without the addition of GM-CSF (concentration: 0.1 ng/mL). Phenotypic analysis was performed using both APAAP and Flow Cytometry (FC), in cells obtained before the suspension (T0h) and after 24h of incubation (T24h). Results: High percentage of granulocytes was positive for HLA-DR (Ia) and CD33 after GM-CSF or G-CSF administration (Table 1).

Table 1. Percentage (%) of la+ and CD33+ granulocytes.

<table>
<thead>
<tr>
<th>Type</th>
<th>LA</th>
<th>CD33</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>25-100</td>
<td>10-95</td>
</tr>
<tr>
<td>ALL</td>
<td>2-83</td>
<td>1-80</td>
</tr>
<tr>
<td>CML</td>
<td>6-40</td>
<td>15-80</td>
</tr>
<tr>
<td>MDS</td>
<td>0-75</td>
<td>20-100</td>
</tr>
<tr>
<td>NHL</td>
<td>15-90</td>
<td>30-98</td>
</tr>
<tr>
<td>MM</td>
<td>0</td>
<td>85</td>
</tr>
<tr>
<td>AA</td>
<td>10</td>
<td>70</td>
</tr>
<tr>
<td>Solid Tumors</td>
<td>10-60</td>
<td>30-90</td>
</tr>
</tbody>
</table>

In most cases those antigens were expressed on granulocytes from the first day of GFs’ administration and they were preserved on their surface even 20 days after the final day of GF injection. In vitro tests showed induction of HLA-DR on granulocytes after incubation with GM-CSF (Table 2). Conclusions. According to our data, administration of growth factors induces the circulation of HLA-DR+ granulocytes in peripheral blood. In vitro tests also confirm this finding. In addition, the results from in vitro tests indicate that GM-CSF directly affects granulocytes, inducing synthesis and expression of the antigen-presenting molecule HLA-DR. Still remains to be proved whether this expression has any functional antigen-presenting role. CD33 is a marker of immaturity and has a regulatory effect in the maturation of myeloid cells, the monocytes/macrophages function and the production of dendritic cells. Its expression on high percentage of granulocytes could be due to: (a) increased mobilization of granulocytes from bone marrow due to CFs’ effect; (b) high rate of differentiation, resulting in preservation of immature markers on granulocytes surface; and (c) possible reactivation of CD33 genes due to cytokines effect.

Table 2. Percentage (%) of la+ and CD33+ granulocytes in vitro tests.

<table>
<thead>
<tr>
<th>Type</th>
<th>LA</th>
<th>CD33</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0h</td>
<td>RPMI</td>
<td>RPMI+GM-CSF</td>
</tr>
<tr>
<td>Healthy</td>
<td>0-2</td>
<td>5-20</td>
</tr>
<tr>
<td>AML</td>
<td>2-5</td>
<td>10-35</td>
</tr>
<tr>
<td>Solid Tumors</td>
<td>5</td>
<td>20-25</td>
</tr>
<tr>
<td>T24h</td>
<td>RPMI+FBS</td>
<td>RPMI+GM-CSF+FBS</td>
</tr>
<tr>
<td>Healthy</td>
<td>0-2</td>
<td>5-20</td>
</tr>
<tr>
<td>AML</td>
<td>2-5</td>
<td>10-35</td>
</tr>
<tr>
<td>Solid Tumors</td>
<td>5</td>
<td>20-25</td>
</tr>
</tbody>
</table>

1458
SEVERE AZATHIOPRINE-INDUCED BONE MARROW APLASIA IN THIOPURINE S-METHYLTRANSFERASE DEFICIENT PATIENT WITH CROHN’S DISEASE

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Thiopurine S-methyltransferase (TPMT) is an enzyme that catalyzes S-methylation of thiopurine drugs, which activity is genetically determined. TPMT-deficient patients are at risk of toxicity after standard doses of thiopurine drugs. There is a large interindividual variability of TPMT activity, mainly due to genetic polymorphism. The three variant alleles: TPMT*2, *3A and *3C are responsible for over 95% cases of lower enzyme activity. Case Report: A 49-year-old male patient diagnosed with Crohn’s disease at the age of 45 was administered azathioprine (AZA) at a daily dose of 2.5 mg/kg. After a month of treatment he developed severe bone marrow aplasia, confirmed by bone marrow biopsy and was hospitalized. Genotyping for TPMT polymorphism (*1, *2, *3A, *3B and *3C) was performed by PCR-based methods and revealed variant homozygous genotype (TPMT*3A/*3A), determining deficiency of TPMT activity. After AZA withdrawal from treatment the patient’s blood cell count started to normalize. He was prescribed methylprednisolone at a daily dose of 24 mg and finally disposed home 17 days afterwards AZA withdrawal, with the following blood parameters: RBC - 2.99 T/L, WBC - 14.9 G/L, PLT - 111.0 G/L. This observation is in concordance with the previous reports, indicating that myelosuppression in TPMT-deficient patients treated with a standard dosage of thiopurines occurs on average 1 month after initiation of treatment. Available data shows, that TPMT-deficient Crohn’s disease patients can be efficiently and safely treated with 5-15% of standard AZA dose (0.16-0.29 mg/kg) or AZA should be replaced by other medication, otherwise it involves severe myelosuppression. In the present report a clear coincidence of AZA administration and severe myelosuppression is documented. In the view of lack of any other potential factors, which might contribute to the observed myelosuppression it can be concluded that accumulation of toxic thioguanine nucleotides in a TPMT deficient patient, was responsible for the observed bone marrow aplasia. For the reasons given above, evaluation of TPMT polymorphism in patients treated with thiopurine drugs should be mandatory in order to optimize therapy.

1459
MUTATION SCREENING IN THE HUMAN ε-GLOBIN GENE USING SINGLE STRAND CONFORMATION POLYMORPHISM ANALYSIS

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Backgrounds. The human ε-globin gene is indispensable for primitive human erythropoiesis. Embryonic ε-globin gene is expressed at high levels early in development in the yolk sac, producing the ε-globin chain, which participates in the formation of the abovementioned Hb tetramers. Although there are over 1,200 different mutant alleles, identified in the human α-, β-, γ- and δ-globin genes, there are no nucleotide
changes in the human ε-globin gene reported to date, rather than a single nucleotide polymorphism (SNP), located at the 5′ regulatory region of the gene. Aims. To develop a non-radioactive single strand conformation polymorphism (SSCP) approach to screen the human ε-globin gene and its regulatory regions for possible mutations and single nucleotide polymorphisms in normal adult subjects, in order to determine those genotypes which are dispensable for its proper regulation and function. Methods. Peripheral blood was collected from 60 unrelated normal male and female donors, whose age ranged between 25-50 years. Informed consent was obtained prior to the study. Genomic DNA was extracted from peripheral blood leucocytes. Human ε-globin gene coding and regulatory regions were amplified in 5 consecutive fragments, using 25 pmole of each primer (primer sequence available on request). PCR products were then analyzed, using a non-radioactive (silver-staining) single strand conformation polymorphism (SSCP) analysis. Where needed, temperature was adjusted (from 4-8°C, increment: 1°C) to improve resolution. DNA sequence analysis was performed using automated fluorescent DNA sequencer (ABI PRISM 510 Genetic Analyzer, Applied Biosystems, CA, USA). Results. Selection of the fragments was done on the basis of analyzing the entire coding and regulatory regions of the ε-globin gene in addition to the majority of intronic sequences. Heterozygous and homozygous cases for the ε/ε/HindII SNP were analyzed as positive controls to ensure optimal resolution and detection of the expected nucleotide changes. Apart from the abovementioned SNP in the expected frequencies for the Hellenic population, our mutation screening approach of 120 normal chromosomes from adult individuals yielded no other nucleotide change in all samples in the region analyzed. DNA sequencing of 10 randomly selected cases in all the fragments confirmed the presence only of the wild-type sequence. A reminiscent of this situation is the human ε-globin genes, which are also mutation-free in their proximal promoter regions. Conclusions. This observation suggests that nucleotide changes in the human ε-globin gene are most likely incompatible with normal erythropoiesis and proper embryonic development. The possibility that mutations or SNPs are present in the 5′2bp region of intron II, which has not included in our experimental design, cannot be ruled out, although it is less likely as this region is also mostly unaltered in the rest of the globin genes studied.

1461 OUTCOME OF CHILDREN WITH APLASTIC ANEMIA IN A DEVELOPING COUNTRY. A SINGLE CENTRE EXPERIENCE

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Background. Aplastic anaemia (AA) is a heterogeneous clinical syndrome, representing a serious challenge for a health system confronted with shortages and with limited experience in bone marrow transplantation. Aims. Given these conditions of treatment we sought to evaluate outcomes of patients with AA treated in our centre. Methods. We retrospectively analyzed the records of 28 patients consecutively admitted in our centre in the period from 1995 to 2005; their mean age was 10.5 years (3 months 22 year) and the sex ratio male/female was 1.33. They all met the criteria for AA: 2 - non severe, 2 - severe and 24 - very severe form. For all patients a diagnostic workup had been performed consisting of: quantitative serum immunoglobulin panel, flow cytometry study of lymphocytes, anti-nuclear and anti-DNA test, viral hepatitis, cytomegalovirus, Epstein-Barr virus, HIV-1 and 2 serologies, bone marrow aspirate and biopsy, including histologic and cytogenetic analyses. Three patients fulfilled the criteria for hereditary AA (Fanconi anemia-1, Dyskeratosis congenita, dominant form -1 case). 6 cases were diagnosed with antithymocyte (ATG) in 10 and Flud with CY and ATG in 3 SCTs. In 12 pts ATG + CsA. With very severe form was transplanted with related HLA-compatible marrow. Most of patients (17-60.7%) died: 8 with severe sepsis, 5 with bleeding accident and 1 developed a fatal myeloblastic leukemia. The unfavourable outcome characterized 3 patients with hereditary form (except the patient with dyskeratosis congenita), all patients with postinfectious AA and the patient with IgA deficiency. 12 cases with idiopathic AA survived, 8 of them with very severe form, treated during the first 2-4 months of disease with ATG + CsA. Conclusions. AA remains in our experience the hemato-

gologic disease with the worst prognosis. To assure the accessibility to allogeneic bone marrow transplantation and to appropriate immuno-

suppressing therapy is our mandatory future task.

1462 ACUTE LEUKEMIA AND MYELODYSPLASIA REVEALING FANCONI ANEMIA: REPORT OF 6 CASES.

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1Hopital Farkhat Hached, SOUSSE, Tunisia; 2Hopital Farkhat Hached, SOUSSE, Tunisia

Fanconia anemia (FA) is a rare autosomal recessive disease characterized by progressive pancytopenia, congenital malformations and predisposition to myelodysplasia (MDS) and acute myeloid leukemia (AML). FA is rarely revealed by AML or MDS. We report 6 cases of patients unknown before with AF and who develope AML and MDS. The ages of patients ranged from 2 to 25 years. The mean age at diagnosis was 11 years. Malformations were present in two cases and consisted of skeletal malformations. Abnormal skin pigmentation were present in 5 cases. AML was noted in 4 cases and MDS in 2 cases. The diagnosis of FA had been proven by chromosome breakage analysis. Cytogenetic analysis showed monosomy 7 in 3 cases and del 6p in one case. The chemotherapy was delivered only in 2 cases. The outcome was unfavourable with death in 5 case. This study suggest to perform systema-

tically a cytogenetic analysis to diagnose FA in childhood AML in tunisian population, which is characterized by its heterogenous ethnic background and by a high rate of consanginiety.

1463 PRE-TRANSPLANT BONE MARROW MICROENVIRONMENT PLAYS AN IMPORTANT ROLE FOR ENGRAFTMENT AND TRANSPANTATION OUTCOME IN NON-MYEOBLASTIE STEM CELL TRANSPLANTATION

Yongsan University College of Medicine, SEOUl, South-Korea

Backgrounds. It has been suggested that both bone marrow (BM) microenvironment and hematopoietic stem cells play important roles
In this study, we tried to evaluate the relevance of BM microenvironment implicated in the engraftment and transplantation outcome after non-myeloablative stem cell transplantation (NST). However, the relevance of BM microenvironment implicated in the engraftment and transplantation outcome after non-myeloablative stem cell transplantation is understudied. We choose patients with moderate-to-severe symptoms of survivors experienced acute/chronic GVHD. Conclusions. Our findings demonstrate that more than half of childhood blood cancer survivors experience different pronounced symptoms in long-term period after transplantation. This confirms the importance of symptom monitoring in order to improve/preserve quality of life in long-term survivors of childhood blood cancer after allogeneic BMT/HSCT.

**INCIDENCE OF HLA ANTIBODIES IN ALLOGENEIC STEM CELL RECIPIENTS**


Medical University of Graz, Austria, GRAZ, Austria

The presence of patient-anti-donor HLA alloantibodies can increase the risk of graft rejection in allogeneic stem cell transplantations and a positive crossmatch against donor lymphocytes may be a predictor for graft failure. Therefore evaluation of patients’ sera for HLA antibodies prior to transplantation is routine in most centres. We additionally focussed on the development of HLA antibodies after HLA fully matched compared to partially mismatched stem cell transplantations and on consequential clinical complications. Sixteen patients who were fully matched with HLA 12 parameters were transplanted and one antigen mismatched allogeneic stem cells were screened for HLA class I and II antibodies by ELISA based methods at the time of registration, prior to and in monthly intervals subsequently to transplantation. Donors were tested for HLA antibodies once prior to transplantation. Cell crossmatchings based on complement dependent cytotoxicity (CDC) were done in 18 cases, depending on the availability of sufficiently viable donor cells. The mean observation period was 84 (28-202) days post transplantation. Two patients and one donor had HLA antibodies before transplantation, which were not directed against transplanted or host antigens respectively, as these transplantations were fully matched. All lymphocytotoxic crossmatches were negative. Pre-formed antibodies were detectable up to day +60 after transplantation. Five patients developed de novo HLA alloimmunization between day +14 and day +112. Three of them had received fully matched and two got HLA mismatched grafts. All alloantibody specificities were unrelated to host or graft HLA antigens. Relapse was reported on 6 of the not immunised and one of the immunised patients. There was no association between the development of HLA antibodies and acute or chronic GVHD. The only patient who rejected his graft had no HLA alloantibodies at all. Among our patients HLA antibodies did not raise a problem in transplantation schedule. Nevertheless we routinely go on evaluating all patients for HLA antibodies whenever a donor search is started in order to define unacceptable HLA mismatches. Immediately before HLA mismatched transplantations patients and donors are screened for antibodies against shared HLA antigens, as donor cells of sufficient viability for crossmatching are not always available. After stem cell transplantation graft-host recognition did not seem predominantly responsible for alloimmunisation in our patients and the development of HLA antibodies was no clear predictive factor for GVHD, relaps or graft rejection.

**SYMPTOMS IN LONG-TERM SURVIVORS OF CHILDHOOD BLOOD CANCER AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT), HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)**

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St. Petersburg State Medical University, ST.PETERSBURG, Russian Federation; Multinational Center of QoL Research, ST. PETERSBURG, Russian Federation; National Pirogov Medical Surgical Center, MOSCOW, Russian Federation

Background. Allogeneic BMT/HSCT improves outcomes in children with blood cancer. However late effects in long-term survivors after BMT/HSCT are understudied. Aims. In this connection the aim of our study was to assess symptoms in long-term survivors of childhood blood cancer after allogeneic BMT/HSCT. Patients and Methods. Eighteen survivors were evaluated at 1-13 years (median, 3 years) after allogeneic BMT/HSCT for acute leukemia (15), chronic leukemia (2) and myelodysplastic syndrome (1). Median age at transplantation was 13 months (range 2 - 21), girls/boys - 11/7. Acute or chronic graft-versus-host disease (GVHD) after BMT/HSCT was observed in 12 survivors. For symptom assessment NH Children Cancer Symptom Inventory and MD Anderson Symptom Inventory were used in the group younger than 15 yrs at the time of the survey (n=11) and in the group 15 yrs and older (n=7), respectively. B- and T cell crossmatches based on complement dependent cytoxicity (CDC) were done in 18 cases, depending on the availability of sufficiently viable donor cells. The mean observation period was 84 (28-202) days post transplantation. Two patients and one donor had HLA antibodies before transplantation, which were not directed against transplanted or host antigens respectively, as these transplantations were fully matched. All lymphocytotoxic crossmatches were negative. Pre-formed antibodies were detectable up to day +60 after transplantation. Five patients developed de novo HLA alloimmunization between day +14 and day +112. Three of them had received fully matched and two got HLA mismatched grafts. All alloantibody specificities were unrelated to host or graft HLA antigens. Relaps was reported on 6 of the not immunised and one of the immunised patients. There was no association between the development of HLA antibodies and acute or chronic GVHD. The only patient who rejected his graft had no HLA alloantibodies at all. Among our patients HLA antibodies did not raise a problem in transplantation schedule. Nevertheless we routinely go on evaluating all patients for HLA antibodies whenever a donor search is started in order to define unacceptable HLA mismatches. Immediately before HLA mismatched transplantations patients and donors are screened for antibodies against non shared HLA antigens, as donor cells of sufficient viability for crossmatching are not always available. After stem cell transplantation graft-host recognition did not seem predominantly responsible for alloimmunisation in our patients and the development of HLA antibodies was no clear predictive factor for GVHD, relaps or graft rejection.
ELEVATED TNF-α AND LDL WITHOUT DISTURBANCE IN PARATHORMONE-ASSOCIATED WITH DIFUSE OSTEOLYTIC LESIONS IN LEUKEMIC TRANSFORMATION OF MYELOFIBROSIS

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1 University of Kragujevac, Kragujevac, Yugoslavia; 2 Institute of Pathology, Belgrade, Serbia and Montenegro; 3 Institute for Molecular Biology, Belgrade, Serbia and Montenegro

Backgrounds. Myelofibrosis is a clonal myeloproliferative disorder characterized by splenomegaly, abnormal deposition of collagen in the bone marrow, extramedullary haematopoiesis, daciecytosis and leukoerythroblastosis. These findings are mediated by complex network of several cytokines. These cytokines mainly include transforming growth factor α, basic fibroblast growth factor, vascular endothelial growth factor, platelet factor 4, calmodulin and tumor necrosis factor α. Aims. Based on role cytokines in myelofibrosis, we present an atypical case of leukemic transformation in myelofibrosis associated with diffuse osteolytic lesions and extremely elevated sera TNF-α and LDL without disturbance in parathormone in a 49-year-old female that firstly developed malaise and abdominal pain at first visit. Results. The laboratory analyses showed decrease in hemo globin, increase in platelets and presence of erythroblast and daciecytosis in peripheral blood. Cytological examination disclosed hypocellularity with the presence of all cell lines without increased blasts cells. Bone marrow biopsy disclosed hypocellularity, presence of all cell lines and bone marrow reticulin and collagen fibrosis. A diagnosis of myelofibrosis was established. After 5 years, her condition deteriorated with malaise and bone pain. Physical examination showed palmar skin and mucous membranes with enlarged spleen 270 mm in diameter. The laboratory analyses showed Hb of 54 g/L, WBC of 8.0×10^9/L, platelets of 122×10^9/L, with myeloblasts 39%, myelocytes 7%, metamyelocytes 1%, bands 6% segmented neutrophils 16%, eosinophils 1%, lymphocytes 22%, monocytes 6%, and 13 erythroblasts/100 leukocytes. The biochemical analyses showed extremely elevated sera LDH activity (1335 U/L). Bone marrow aspirate was hypocellular with 72% of blasts mostly with characteristics of myeloblasts and more than 20% of monoblastic type. Cytochemical staining with myeloperoxidase showed that 30% of blasts were positive, and 28% of blasts were α-naphthol-esterase positive. The cytological finding was in accordance with FAB M4 type of acute leukemia. The immunophenotyping of the peripheral blood cells expressed HLA-DR (74.96%), CD34 (77.99%), CD13 (60.36%), CD33 (42.60%), CD14 (39.89%), CD4 (42.40%) markers. Cyto genetic examination of bone marrow cells showed inversion of chromosome 16 (t(16;16), inv(16)(p13q22)). RT-PCR studies MYH11 fusion gene confirmed cytogenetic finding and revealed the CBF transcript. PCR analysis disclosed the presence of FLT3 Asp835 mutation. Retrospective analyses of extracted DNA from bone marrow histological specimen at the time of diagnosis, showed that there no presence of FLT3 mutations. X-ray showed the presence of diffuse osteolytic lesions in the pelvis, long bones as well as in vertebrae. The global laboratory parameters documented diffusely increased accumulation of the radiopharmaca. The values of parathormone in the sera and supernatants of cultured blast cells were normal. TNF-α determined by sensitive LDH release assay, was extremely increased (1421 pg/mL) in comparison to control values of 700 pg/mL. Summary / Conclusions. We postulated that elevated TNF-α can be reason for lytic bone lesions, accompanied with high sera LDH activity indicating high bone turnover. Also continuously elevated TNF-α can contribute for developing of the leukemia growth in this patient, as endogenous promoter.
M.O. Freitas, | haematologica/the hematology journal | 2006; 91(s1)

### 1470 MIXED IMMUNEDEFICIENCY, ATYPICAL MYCOBACTERIA AND MYELOFIBROSIS

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**Background and Aims.** Myelofibrosis can be idiopathic (a chronic myeloproliferative syndrome) or secondary to many kinds of insults, as a reaction to malignancy, infections, endocrinopathies, autoimmune diseases, and hematopoietic or hepatosplenomegaly are frequent, and only reversible when the secondary injury can be treated. The authors present a case of myelofibrosis diagnosed in an 11 months old boy; later it was discovered to be secondary to atypical mycobacteria and quadruple antibacterial therapy for one year reversed the clinical status. The boy was given G-CSF on alternate days (10 µg/kg), immunoglobulin on a mensal basis and co-trimoxazole as prophylaxis for infections. With two years of age, he doesn't have a significative number of bacterial infections. The spleen varied between 1, 5 and 2, 5 cm under the costal grid, his physical and somatometric development were normal for his age. The clinical status degrades with a growing splenomegaly and pancytopenia. In the relation CD4+/CD8+ and auto antibodies anti-platelets and anti-granulocytes. The boy was given G-CSF on alternate days (10 µg/kg), immunoglobulin on a mensal basis and co-trimoxazole as prophylaxis for infections. With two years of age, he doesn't have a significative number of bacterial infections.

**Methods.** Aims. Myelofibrosis can be idiopathic (a chronic myeloproliferative syndrome) or secondary to many kinds of insults, as a reaction to malignancy, infections, endocrinopathies, autoimmune diseases, and hepatosplenomegaly are frequent, and only reversible when the secondary injury can be treated. The authors present a case of myelofibrosis diagnosed in an 11 months old boy; later it was discovered to be secondary to atypical mycobacteria and quadruple antibacterial therapy for one year reversed the clinical status. The boy was given G-CSF on alternate days (10 µg/kg), immunoglobulin on a mensal basis and co-trimoxazole as prophylaxis for infections. With two years of age, he doesn't have a significative number of bacterial infections.

**Results.** The spleen varied between 1, 5 and 2, 5 cm under the costal grid, his physical and somatometric development were normal for his age. The clinical status degrades with a growing splenomegaly and pancytopenia. In the relation CD4+/CD8+ and auto antibodies anti-platelets and anti-granulocytes. The boy was given G-CSF on alternate days (10 µg/kg), immunoglobulin on a mensal basis and co-trimoxazole as prophylaxis for infections. With two years of age, he doesn't have a significative number of bacterial infections. The spleen varied between 1, 5 and 2, 5 cm under the costal grid, his physical and somatometric development were normal for his age. The clinical status degrades with a growing splenomegaly and pancytopenia. In the relation CD4+/CD8+ and auto antibodies anti-platelets and anti-granulocytes. The boy was given G-CSF on alternate days (10 µg/kg), immunoglobulin on a mensal basis and co-trimoxazole as prophylaxis for infections. With two years of age, he doesn't have a significative number of bacterial infections.

**Conclusion.** Functional disorder of the platelets seems to be the part of clinical findings of the disease, but does not correspond with biological activity of the disease or with its clinical symptoms and/or with the answer to the therapy. Although, the treatment (especially with acid acetylsalicylic-ASA) can widely modify platelet function, it was not even observed to be significantly different in our ASA-treated vs. ASA-untreated patients.

### 1471 PLATELET FUNCTION EXAMINATION IN ESSENTIAL THROMBOCYTHYMIA


**Background.** Basic diagnosis of essential thrombocythemia is proved by estimation of elevated platelet count and corresponding findings of an increased megakaryopoiesis in bone marrow with progressive and immature megakaryocytes finding imatinib treatment was started. Imatinib in dose of 100 mg daily was administered despite the F/P negativity. Results. Eosinophils fully disappeared after 6 days of the therapy. Complete hematologic remission was achieved after 2 weeks. Cytogenetic response was achieved after 3 months (Table I).

**Results.** The platelet aggregation was tested using ADP in 16 cases after all inductors used was not accompanied by statistically significant changes in other examinations of platelet function tests. Moreover, there were not observed statistically significant changes in repeated examinations after six month of the treatment. There was even a no correlation between functional examination of the platelets and clinical symptoms of the disease. Conclusion: Functional disorder of the platelets seems to be the part of clinical findings of the disease, but does not correspond with biological activity of the disease or with its clinical symptoms and/or with the answer to the therapy. Although, the treatment (especially with acid acetylsalicylic-ASA) can widely modify platelet function, it was not even observed to be significantly different in our ASA-treated vs. ASA-untreated patients.

### 1472 POLYCYTHEMIA VERA INITIALLY DIAGNOSED AS ESSENTIAL THROMBOCYTHYMIA

M. Badea, D. Badea

**Background.** The differentiated diagnosis as part of the myeloid proliferative chronic disease Ph- remains afterwards difficult, in spite of the WHO, because of the many similarities and the various clinical and biological parameters (ex. WHO). Aims. The aims of this piece of work are to present our experience concerning the difference between ET and PV at diagnosis. Method: The study contains a number of 38 patients with PV diagnosed in our clinic during the period of 1998-2005. 5 of those cases whose age was 46, 54, and 60 years old were diagnosed initially with ET. Results. The value of Hb was 15, respectively 16.5g/dl with Ht 44 respectively 46% for the 2 female cases and 17g/dl respectively 51% for male patients. 2 patients presented at diagnosis, leucocytes under 10,000/mm³, only a case from the three of them presented more than 12,000/mm³. The spleen varied between 1, 5 and 2, 5 cm under the costal board. At the beginning, the value of Hb and Ht did not allow the diagnosis of PV and the high count of the platelets (650 0000, 74 0000 and 82 0000/mm³) imposed the diagnosis of ET. The bone marrow examination was applied (after 2002) for only a patient, releasing on bone marrow biopsy: hypercellularity with hyperplasia of all marrow elements, with left deviation of the erythrocyte clusters, a polymorph megakaryocytic aspect, and that's why it was considered unclassified myeloproliferative disorder. The evolution of those three patients was in 8-18 months towards a classic PV with high levels of Hb and Ht who needed phlebotomy. Conclusions. At the point of prognostic and the therapeutic approach for these 2 chronic diseases. Recently the difference becomes more important at point of molecular view (TH V617F), even possible prognostic (ET after 10 years of evolution). The histological
study of the marrow and the evaluation of the new biological markers can chunk the diagnosis even at the beginning of the diseases

1473
SUCCESSFUL TREATMENT OF CHILDHOOD IDIOPATHIC MYELOFIBROSIS WITH STEROID
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Idiopathic myelofibrosis can develop in children as well as adults. However, the disease seems to be different from adults requiring a more conservative approach to management. It is less commonly seen than adults and appears to be less aggressive, being characterized by a variable outcome reported in literature from aggressive course and a high mortality to less aggressive course and even spontaneous regression. We reported the case of idiopathic myelofibrosis in the early childhood of a boy successfully treated with standard doses of steroid. A 4 month-old boy was admitted because of severe anemia with reticulocytopenia, aniso-poikilocytosis, leukoerythroblasts, teardrop-shaped red cells and splenomegaly. The marrow was very difficult to aspirate. The bone marrow biopsy revealed reticulin myelofibrosis. Cytopathenic study of the marrow was negative. The condition worsened with development of severe thrombocytopenia. Investigations done repeatedly ruled out malignant hemopathy, metastatic infiltration of the marrow, myelodysplasia, osteopathy, lupus erythematosus, immune disease and Fanconi anemia. After 5 months and 3 red cells transfusion prednison therapy was attempted at 2 mg/kg/d. A complete improvement of hematological and clinical findings was observed after a month and a half. He is now 13 months old on 1.5mg/kg/d of steroid with a hemoglobin of 14g% and platelets around 410000/mm³.

1474
QT DISPERSION AFTER ADMINISTRATION OF RAPID INTRAVENOUS VARIOUS ANTIEMETICS IN CHILDREN PRIOR TO CHEMOTHERAPY
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Backgrounds. Children with acute leukemia are at an increased risk of cardiac arrhythmias, from their cardiac infiltrations and cardiotoxic treatments. There are many reasons why the children with acute leukemia are at increased risk of potentially life-threatening cardiac arrhythmias. The autonomic response to chemotherapy and radiation therapy (nausea, retching and vomiting) or their biochemical effects (vomiting-induced electrolyte disturbance) can have important implications. It is, therefore, important to ensure that any medications to-administered to the children, do not further increase the risk of cardiac complications, particularly arrhythmias. Nausea and vomiting are considered to be the most distressing and debilitating side effects of therapy, and can profoundly affect patients’ quality of life. Aim and Methods. The aim of this study was to determine the effect of the rapid administration of intravenous tropisetron, granisetron and ondansetron on measures of cardiac depolarization in children receiving chemotherapy for acute leukemia, by comparing twelve-lead ECGs before (baseline) and after 2nd and 24th hours after the drug administration. Results. The study was performed in total 75 children with acute leukemia (25 children for each antiemetic). QT dispersion was calculated as the difference between the maximum and minimum QTc in twelve-lead surface electrocardiogram lead. Summary/Conclusions. It was concluded that no clinically important cardiac distinctions in quantities autoblood (795,0±21,7 and 906,7±45,8 mL, p<0,05), red autoblood cells (354,3±94,5 and 881,2±82,6 mL, p<0,05) and washed erythrocytes (295,6±122,2 and 611,4±93,7 ml, p<0,001) between groups 1 and 2 are revealed. Conclusion: Use rtVIIa in therapy of uncontrollable not surgical bleedings at CPB results in significant reduction of frequency of use and volumes blood transfusion.

1476
ANALYSIS OF THE EFFECTIVE AGENTS IN HEPATITIS C VIRUS INFECTION AMONG HEMOPHILIC PATIENTS TREATED IN HEMOPHILIA CENTER OF ISFAHAN-IRAN
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Backgrounds. Patients with hemophilia are at high risk of post-transfusion hepatitis because of widespread use of plasma-derived products. As a consequence, hepatitis C virus (HCV) is the most common cause of chronic liver disease among hemophiliac patients. Aim: The objectives of this study are to determine HCV prevalence, and analyze the effective agents in HCV infection in hemophiliac patients. Methods: all patients with inherited coagulation disorders registered in hemophilia center of I.R.I (535 persons) were checked for HBsAg and anti-HCV, using a third-generation enzyme-linked immunosorbent assay (ELISA) test. Positive tests for anti-HCV were confirmed by RT-PCR. Clinical history, Laboratory and treatment data of all cases were studied in January 2006. Results. From 465 men and 88 women with inherited coagulation disorders with Mean±SD age of 23.4±12.9 years, 125 patients (22.6%) were HCV positive, 2 (0.4%) were HBV positive and one(0.2%) was both HBV and HBV positive. In this study the chance of coloration (with percentage correct of 72.4%) between HCV infection and cryoprecipitate usage was 5.51, between HCV and FFP usage was 3.18 and between HCV infection and moderate and severe hemophilia were 3.9 and 2.65 respectively. In this study blood group, factor concentrate consumption, age and sex of patients have no predictive value in HCV infection. 44.4% of patients with factor inhibitor were HCV positive. (p=0.006). Conclusion: Considering the high chance of HCV infection after transfusion of Cryoprecipitate and FFP, a more careful pre-transfusion screening of blood for anti-HCV must be introduced in all blood banks. The usage of FFP which has less chance of HCV infection, instead of cryoprecipitate in patients who do not have volume restrictions may be preferable.

1477
CLINICAL AND EPIDEMIOLOGICAL CHARACTERISTICS OF THROMBOCYTOPATHIES IN CHILDREN IN KAZAKHSTAN
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The problem of bleedings in childhood is one of the most actual. Thrombocytopenias (TP) - the group of diseases characterized by platelet dysfunction with predominantly microvascular type of bleeding. In structure of hemorrhagic diatheses TP has leading position (80%). The aim of this study was to assess the clinical and laboratory peculiarities of TP in 105 children hospitalized in hematomatological department of the Scientific center of pediatrics and children’s surgery. Diagnostic complex was included anamnestic data, duration and character of the bleeding, platelet number and coagulogramm. Genealogical anamnesis on bleeding was aggravated in 75% of patients with predominantly autosomal-dominant inheritance. In 15 patients parents were examined. Hereeditary TP was diagnosed in 95 (91%) patients, acquired TP in 10 (9%), out of them: drug-induced in 4 (4,5%), post-infectious in 4 (3,5%), due to endocrinopathy (hypoestrogenia) in 2 (1%). Hemorrhagic syndrome was primarily diagnosed in earlier childhood in 57 patients (54%), frequency of the relapses was increased to 10-12 y.o. in 77 patients (73%), to 15-16 y.o. bleeding symptoms regressed. Patients were divided into groups: I group - with deficit of plasma adhesion and aggregation factors (von Willebrand disease, vWD and asibrinigenemia, aF) and II group - with platelet factors alterations (inherited TP). Patients of the I group had complaints: nasal bleedings (80%) and skin hemorrhages (54%). Patients of the II group had less complaints: nasal bleedings (58%)

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or skin hemorrhages (24.3%), in 43% children were directed by other specialists due to petechiae appearing after procedures, accidents or others. Perinatal pathology (skin hemorrhagic syndrome, umbilical hemorrhages, intranatal intraventricular hemorrhages) was more frequent in I group (54.5%) in comparison with II group (27.3%). Bleeding time was increased in 98.8% of patients. Alterations in adhesive and aggregative platelet functions were noted in 57.4% (istocitin-induced platelet aggregation decreased in 55% of patients, adrenalin-induced aggregation in 7 to 7-8 µm were noted in 8.1% of patients. In 30 patients changes in coagulogramm were observed: alteration of APTT, vWF activity decreasing (lower than 75%) and plasma vWF level (lower than 80%). In 62.6% alterations in platelet aggregation were observed: in 52 patients - an absence of collagen-induced platelet aggregation, in the rest - adenosine-diphosphate (ADP), adrenalin-induced aggregation decreased. Prominent alteration of clot retraction was revealed in 11 patients. Thus, dysfunctions in primary chain of hemostasis in our patients were predominantly inherited, and clinical manifestation was comparatively more severe. In structure of hemostasiopathies thrombocytopathies of releasing (52.2%) and von Willebrand disease (29.2%) were prevailed, which, possibly, can be explained by dominant inheritance. Any signs of bleeding diatheses in children are should be approached and require specialized investigations, including examination of relatives with aggravated anamnesis on bleeding.

1478 NASAL BLEEDING IN CHILDREN WITH HEREDITARY THROMBOCYTOPATHIAS
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Nasal bleeding in children is not rare pathological condition, which causes diagnostic and therapeutic difficulties among physicians. Bleeding from nasal cavity is not a disease, but the symptom of the local or systemic disease. The most intensive and severe nasal bleeding more often has place in cases of hereditary thrombocytopathia. Hemorrhagic diatheses are characterized by hereditary, congenital or acquired system bleeding disorders. According on data of various investigators, from 40% to 80% of all cases of bleeding disorders related with quantitative and qualitative disorders of thrombocytic hemostasis. Among thrombocyte-dependent hemostasis disorders the special interest is directed to thrombocytopathies, because fact that 80% of hemorrhagic diatheses are related with disorders of primary (thrombocyctic) stage of hemostasis. However, the clinical manifestations of the majority of hemostasiopathies are monotypic, which makes the diagnostic difficulties. Earlier establishment of the cause of hemostasis disorder is necessary for administration of an adequate hemostatic therapy. Under our observation were 42 children aged from 1 to 15 years old with thrombocytopathies, hospitalized to oncopharmacologic center in SCPCS. The diagnosis was based on anamnesis, clinical manifestation, laboratory data. Assessment of hemostasis system was based on the results of standard investigations: platelet number, time of bleeding, coagulation time, prothrombin time, activated partial thromboplastin time, blood fibrinogen level, von Willebrand factor. Generally, all those indicators was in normal ranges. An assessment of clot retraction, the decreasing (lower than 40%) was noted in 4 patients, and Glanzman’s thrombaste- nin was established in 38% of patients. The diagnosis of von Willebrand disease was established in 26% of patients with normal platelet number, increased activated partial thromboplastin time (over 55 sec), decreased activity of von Willebrand factor (lower than 80%). Neurologist has established vegetative vascular dystonia and intracranial hypertension in 12 patients. In 11 patients otolaryngologist has revealed during the rhinoscopy the superficial localization of blood vessels, especially in Kisselbakh zone. Thus, nasal bleedings in children with thrombo- cytopathias - the most often symptom. Bleeding in form of petechias, ecchimoses, gingival, nasal bleeding in children with normal or slightly decreased platelet number may be due to qualitative disorders of platelets. Bleeding in form of hematomas, hemarthroses reveals double defect in hemostasis, typical for von Willebrand disease. After establishment of bleeding type laboratory investigations to reveal character of hemostasiopathy is necessary. Vegetative vascular dystonia, intracranial hypertension, vascular changes in nasal mucosa, possibly, may play role in etiopathogenesis of nasal hemorrhages in patients with hemostasis disorders. Earlier establishment of the cause of nasal bleedings will allow to conduct an adequate therapy.

1479 DERANGEMENT OF HAEMOSTATIC PROTEINS IN HCV CIRRHOTIC PATIENTS: RELEVANCE TO HEMORRHAGIC DIATHESIS AND THROMBOTIC EPISODES
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Backgrounds. An altered coagulation profile resulting in decreased natural anticoagulant levels leading to haemostatic activation is described in patients with liver cirrhosis. The protein C system, a major physiologic regulator of haemostatic balance, controls thrombin production and guards against thrombotic episodes. Aims. This study was designed to assess the components of protein C system in HCV cirrhotic patients and to determine whether these alterations in the protein C system are related to degree of hepatic dysfunction and/or haemostatic activation and development of hemorrhagic diathesis or thrombotic episodes. Methods. Components of protein C system (PC) were assessed in 44 cirrhotic patients with hepatitis C virus liver cirrhosis, of whom 15 patients had acute hematemesis and 14 patients had portal vein thrombosis (PVT). According to Child-Pugh criteria, all patients were graded Child C. Neutrophil elastase (NE) release was determined by measuring elastase-α1-protease inhibitor (E-α1-PI) complex using an immune activation assay. Levels of tumor necrosis factor-α (TNF-α), PC antigen (PC.Ag), total protein S (TPS), free protein S (FPS), soluble thrombomodulin (TM), tissue-plasminogen activator (t-PA), t-PA-PAI-1, plasmin-α2-antiplasmin (PAP), thrombin-antithrombin III (TAT) and D-dimer (D-D) complexes were measured in plasma by ELISA. Fibrinogen level, functional activities of PC (PC.Ft), plasminogen activator inhibitor-1 (PAI-1) and C4b-binding protein (C4b-BP) concentrations were also assessed. Results. Stimulation of the inflammatory process (increased TNF-α, NE and C4b-BP), endothelial injury (elevated TAT and t-PA), reduction in anticoagulant proteins (low PC and PS), hypercoagulation and thrombin generation (elevated TAT and D-D), increased consumption (prolongation of coagulation screening tests, thrombocytopenia, hypofibrinogenemia and decreased PC. Fv/PC Ag ratio) and accelerated fibrinolysis (increased PAP, free t-PA and t-PA/PAI-1 ratio and decreased PAI-1) were detected in different cirrhotic groups compared to controls (15 healthy subjects). The haemostatic defects correlated with the marked elevation of inflammatory mediators and were more pronounced (p<0.05) in patients with PVT. A significant decline (p<0.05) in fibrinogen concentration and PC Fv/PC Ag ratio associated with a significant increase (p<0.05) in TAT and D-D level in patients with PVT compared to cirrhotics with haemostatic balance. Moreover, FPS and PAI-1 levels were significantly elevated (p<0.05) in patients with PVT compared to those with acute hematemesis and were...
inversonly correlated with platelet count (p<0.01). Conclusions. These findings suggest that NE and TNF-α contribute to haemostatic alterations in patients with viral hepatitis C liver cirrhosis, and emphasize the clinical significance of protein C as a sensitive parameter for hepatic dysfunction and protein S and PAI-1 as reliable prethrombosis markers in these patients.

1480

FX DEFICIENCY IN A GREEK FAMILY

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FX deficiency is a hereditary disease and is one of the rarest factors deficiencies with incidence for homozygous type 1/1.00.000 in the general population. Only 50 cases have been reported all over the world. People can suffer from bleeding when the FX level is below 10%. The symptoms vary from mild to severe bleeding. The heterogeneous type is commonly asymptomatic. A young patient 17 years old investigated in our laboratory in order to take oral contraceptives because she had prolonged bleeding during menstruation. We found slightly prolonged PT and APTT and she was further investigated for TT, Fibrinogen, DDimers, FDP’s, full blood count and full biochemical tests. Her parents and her three brothers and the sister of her father and her 2 children were also investigated. Results. The tests of the patient PT:16.3 sec (INR:1.5), APTT:40.2sec. Then the plasma was diluted 1/1 with normal plasma and then was measured immediately and 1 and 2 hours after incubation in 37°C waterbath. In all the measurements the tests were in normal range and the measurement of the factors FVII, FIX, FX, FXI,FV,WVF is normal except the FX: 46.4% (normal range 70 - 140%). The mother of the patient was normal of all tests. The father had PT:15.1sec, INR:1.24, APTT:38.6sec and FX: 50.5%. The two brothers were normal of all the tests. The third brother had PT:16.2sec, INR:1.33, APTT:42.9sec and FX: 36.8%. The sister of the father had PT:14.3sec, INR:1.11, APTT:37.9sec and FX: 55.6%. From her children the first was normal and the second had PT:15sec, INR:1.33, APTT:37.8sec and FX: 54.9%. The human FX gene is located on chromosome 13q34 and it is an ‘autosomal recessive’ disorder. The half life of the factorX is 40-45 hours. The treatments for control bleeding is FFP and Prothrombin Complex Concentrate.

1481

THE INFLUENCE OF STORAGE AND LEUKOCYTE DEPLETION ON THE ANTIGEN DENSITY IN DUFFY AND MNS BLOOD SYSTEMS MEASURED BY FLOW CYTOMETRY

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Backgrounds. Red blood cell (RBCs) antigens are polymorphic structures located in the RBC membrane. Fya and Fyb antigens are the most important ones in the Duffy system and they are carried by multipass transmembrane glycoproteins. S and s antigens, belonging to MNS system, are carried by glycophorin B (GPB). These RBC antigens are sensitive to enzymes produced by leukocytes, and therefore can present changes in expression during the storage period. The behavior of erythrocyte antigen expressions during storage period is an important marker in the quality control of RBC reagents used in Transfusion Centers. These data can also help to define the RBC reagents validity time. Aims. to evaluate the influence of leukocyte enzymes and storage in the expression of Fya, Fyb, S and s antigens of RBC units collected with CPDA-1. Methods. We studied 49 RBC units, which had been divided into two sub-units immediately after blood collection. One was submitted to a leukocyte reduction using SepacellTM filters before storage and the other was used as a control. Evaluation of antigens was carried out on days 1 and 35 of the storage period using hemagglutination techniques (tube and gel tests) and immunophenotyping by flow cytometry. The determination of the number of antigenic sites for each antigen studied was performed by flow cytometry (FCM) using the QIFIKITTM for standardization. Results. Concerning the influence of leukocyte depletion, only Fya presented an increase in the antigen fluorescence intensity (p=0.02) on day 35 of storage in the leukocyte-depleted bag. The other antigens (Fyb, S and s) presented no difference of expression after leukocyte-reduction. The storage period of 35 days did not affect the antigen density of Fya, Fyb, S and s. Concerning the number of antigenic sites, Fya showed a median number of 1.1 and 2.5 x 10^4 in donors Fy(a+b-) and Fy(a+b+) respectively; Fyb 0.72 and 0.78 x 10^4 in donors Fy(b+b-) and Fy(b+b+); S 1.0 and 2.1 x 10^4 in individuals Ss and ss and antigen s showed 1.0 and 2.4 x 10^5 sites in donors Ss and ss respectively. Conclusion. FCM showed to be an efficient technique, able to detect antigen small expression alterations that were imperceptible with other methods. This technique was more reproducible, stable and appropriate for Fya and Fyb antigens than for S and s antigens, probably due to the characteristics of the commercially available anti-S and anti-s antibodies. The leukocyte-reduction only influenced Fya antigen on day 35 of storage.

1482

COMPARATIVE STUDY OF THREE CD34+ CELL SELECTION DEVICES IN AUTOLOGOUS STEM CELL TRANSPLANTATION SETTING: ISOLEX 1.2 (BAKER), ISOLEX 2.5 (BAKER) AND CLINIMACS (MILTENYI)

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In the setting of autologous stem cell transplantation, several studies suggest tumour-free grafts may improve outcome. Positive CD34+ selection of PBSC (peripheral blood stem cell) can be used as a purging strategy, reducing tumor cell contamination of the graft. To compare purging efficiency and recovery data obtained with three distinct devices. We compared the results of 46 CD34+ cell positive selection processes performed in our center with three distinct devices: Isolex 1.2 (9 cases), Isolex 2.5 (18 cases) and CliniMACS (19 cases). There was no statistical difference between the three groups neither in terms of number of total nucleates cells, number of total CD34+ cells nor in percentage of CD34+ cells. The characteristics of the CD34+ selection column (purity and recovery) obtained with each system were the following: 1) Purity (%): Isolex 1.2: 90.21 (67.5 - 99.3), Isolex 2.5: 97.98 (99.4 - 94.0) and CliniMACS: 97.10 (95.0 - 98.8); 2) Recovery (%): Isolex 1.2: 42.58 (32.93 - 67.80) Isolex 2.5: 65.59 (43.78 - 89.33) and CliniMACS 59.96 (39.96 - 79.96). We compared purity and recovery obtained by the three immunoselection devices (Table 1). In our experience, with the system Isolex 1.2 we obtained a statistically significant poorer recovery and purity of the CD34+ enriched cells as compared with the other two devices. We did not find significant differences between version 2.5 of the Isolex and the CliniMACS, neither in the purity of the final product, nor in the recovery of cells CD34+.

Table 1.

<table>
<thead>
<tr>
<th>Purity</th>
<th>Recovery</th>
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<tr>
<td>Isolex 1.2 vs Isolex 2.5 (p&lt;0.009)</td>
<td>Isolex 2.5 vs CliniMACS (p&lt;0.001)</td>
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<tr>
<td>Isolex 1.2 vs CliniMACS (p&lt;0.031)</td>
<td>Isolex 2.5 vs CliniMACS (p&lt;0.05)</td>
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<tr>
<td>Isolex 2.5 vs CliniMACS (p&lt;0.141)</td>
<td>CliniMACS 2.5 vs CliniMACS (p&lt;0.127)</td>
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1483

ERYTHROCYTE EXCHANGE BY FRESENIUSCOM TEC TO TREAT ACUTE PAIN CRISIS IN SICKLE-CELL DISEASE. A CASE REPORT

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Background and Aims. homozygote patients for sickle-cell disease (SCD) have abnormal hemoglobin which undergoes gradual reversible polymerization and aggregation. Repetition of this process may lead to irreversible membrane changes and characteristic sickle cell morphological changes. In most patients with SCD, bone marrow erythropoiesis is necessary to produce large amounts of normal erythrocytes. Despite TREC has never been shown to influence the expression of the gene for the SCD, it seems a reasonable approach to treat severe acute SCD complications. Furthermore, TREC presents several advantages over direct transfusion increasing HbA

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quickly with concomitant HbS removal and ameliorating hyperviscosity. We report about SCD acute painful crisis in a 25-years-old woman, who benefit by automated TREX performed at our Apheresis Service using Fresenius™.COM.TEC device with a new dedicated program.

<table>
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<th>Table 1. Characteristics of the patient.</th>
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<tr>
<td>1st TREX</td>
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<td>Pain (black, abdominal, legs)</td>
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<tr>
<td>Morphine</td>
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<tr>
<td>Hct (%) / Hb (g/dL)</td>
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<td>Hbs (%)</td>
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<th>Table 2. Characteristics of erythroexange.</th>
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<tr>
<td>1st TREX</td>
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<tr>
<td>No./total volume of RBCs* (mL)</td>
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<tr>
<td>Mean Ht(%) of RBCs* (mL)</td>
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<tr>
<td>Patient blood volume (mL)</td>
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<tr>
<td>Processed blood volume (ML)</td>
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<tr>
<td>Anticoagulant to the patient (mL)</td>
</tr>
<tr>
<td>Flow rate (mL/min)</td>
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<tr>
<td>Time of procedure (min)</td>
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RBCs* = red blood cell units

Methods: A blood specimen was drawn in advance to assess compatibility, then cross-matched RBC units were filtered for leukocyte depletion. A detailed informed consent was obtained. Erythroexange was performed by double-vein technique using a new program (Fresenius HemoCare™, Bad Homburg, Germany) which permits to predict both the final hematocrit and HbS level of the patient. Blood cell count and HbS percentages by current haemoglobin electrophoresis were measured before and after each TREX. The pre-apheresis HbS value permitted to appropriately set the cell separator program with the goal of reducing HbS to less than 30%. Check of post-apheresis HbS allowed verifying the accuracy of instrument predictions. During the procedure the patient was carefully monitored as respect to vital signs (blood pressure, heart rate, oxygen saturation) and occurrence of adverse events, in particular signs of transfusion reaction. Calcium gluconate was administered i.v. to prevent or minimize citrate toxicity. Results: we performed two TREX procedures on alternate days; the relevant data are given in the table 1 and 2. Complete clinical remission was obtained with no evidence of alloimmunization or other serious complications. Conclusions: our experience confirms the beneficial effects of TREX for SCD pain crisis especially when isovolumetric procedures are carried out with an automated device.

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<th>Table 1.</th>
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<td>CD34+                       &gt;0.2%                       &lt;0.2%</td>
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<tr>
<td>Adults                      &gt;20/µL                       97% (104/107)               76% (23/30)</td>
</tr>
<tr>
<td>&lt;20/µL                       82% (14/17)                13% (7/53)</td>
</tr>
<tr>
<td>Children                    &gt;20/µL                       95% (20/21)                92% (12/13)</td>
</tr>
<tr>
<td>&lt;20/µL                       83% (5/5)                  41% (5/12)</td>
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Thrombotic thrombocytopenic purpura (TTP) is a rare complication of hematopoietic stem cell transplantation (HSCT): the literature is scant and heterogeneous, little is known about the pathogenesis, except that it appears to differ from that of classical TTP. Plasma exchange (PE) is commonly employed for the therapy, but there are no data that support its use. We present our experience in treatment of two post-HSCT TTPs with PE. From May 2004 to December 2005, 52 patients underwent HSCT, and TTP was diagnosed in 2 of them, respectively on post transplant day 47 and 102. Both patients received HSCT from HLA-compatible related donors. TTP was defined as the simultaneous occurrence of red cell fragmentation, laboratory findings of haemolysis with negative direct and indirect antiglobulin test, high LDH level, red cell transfusion requirement and thrombocytopenia caused by consumption, in the absence of disseminated intravascular coagulation. PEs were performed using fresh frozen plasma as replacement fluid. PE was well tolerated, but the two patients had no response to the treatment. One patient died because of fungal infections. Our experience confirm the data of the recent literature. TTP is a rare and serious complication of hematopoietic stem cell transplantation and further, systematic studies are necessary for a better knowledge of its incidence, treatment and outcome.

1484
PREHARVEST PREDICTIONS OF STEM CELL PRODUCTS
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Backgrounds. Collected mobilized peripheral stem cell is the commonly used resource for autologous transplantation. The amount of CD34+ cells in the peripheral blood is used as determinant for starting collection. Aims. Aim was to see if not only the amount of CD34+ cells /µl determines the yield of the product but also the percentage of CD34% cells should be considered when to start with collection. Methods. 259 aphereses of adult patients suffering from hematological (AML, CLL, NHL, MM) disorders and children with solid tumors (ewing sarcoma, neuroblastoma, rhabdomyosarcoma,) were evaluated. Aphereses were done with the Cobe Spectra™, 3-4 times the blood volume was performed. Measured were WBC, MNC and CD34+ Cells in the peripheral blood, amount of CD34+ cells /kgBW in the aphereses products, collected CD34+ cells/processed liter and efficacy of the procedures (MNCs, CD34+ cells.) Results. According to the specifications of the transplanting departments (CD34+ cells > 2x10^6/kgBW + back up) 190 (73,85%) of the aphereses were completed successfully, 31 out of 190 (16,31%) successfully completed aphereses were started with CD34+cells<20/µL. In 19 out of 23 aphereses we were successful with CD34+cells<20/µL and >0.2%. Conclusion: In collections started with CD34+cells<20/µL the percentage of CD34+ cells is a good predictive factor for successful apheresis, even more so for adults then for children. So don’t forget to look at the percentage of CD34+ Cells in peripheral blood when you decide to start with stem cell apheresis.

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THROMBOTIC THROMBOCYTOPENIC PURPURA POST-HEMATOPOIETIC STEM CELL TRANSPLANTATION
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Thrombotic thrombocytopenic purpura (TTP) is a rare complication of hematopoietic stem cell transplantation (HSCT): the literature is scant and heterogeneous, little is known about the pathogenesis, except that it appears to differ from that of classical TTP. Plasma exchange (PE) is commonly employed for the therapy, but there are no data that support its use. We present our experience in treatment of two post-HSCT TTPs with PE. From May 2004 to December 2005, 52 patients underwent HSCT, and TTP was diagnosed in 2 of them, respectively on post transplant day 47 and 102. Both patients received HSCT from HLA-compatible related donors. TTP was defined as the simultaneous occurrence of red cell fragmentation, laboratory findings of haemolysis with negative direct and indirect antiglobulin test, high LDH level, red cell transfusion requirement and thrombocytopenia caused by consumption, in the absence of disseminated intravascular coagulation. PEs were performed using fresh frozen plasma as replacement fluid. PE was well tolerated, but the two patients had no response to the treatment. One patient died because of fungal infections. Our experience confirm the data of the recent literature. TTP is a rare and serious complication of hematopoietic stem cell transplantation and further, systematic studies are necessary for a better knowledge of its incidence, treatment and outcome.

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QUALITY OF LIFE OF ONCOLOGICAL AND HEMATOONCOLOGICAL PATIENTS AFTER THE HSCT: FINDING FROM CROSS-SECTIONAL AND RETROSPECTIVE STUDY
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Backgrounds. The cross-sectional, retrospective and descriptive study evaluates quality of life (QoL) of patients after the hematopoietic stem cell transplantation (HSCT) at the Department of Clinical Hematology of the 2nd Internal Clinic of the University Hospital and Medical Faculty of Charles University in Hradec Královy, Czech Republic from 2001 to 2003. Aims. 1. to verify the applicability of the Czech version of an international generic European Quality of Life Questionnaire - Version EQ-5D for the evaluation of QoL in patients after the HSCT at the Department of Clinical Hematology of the Second Internal Clinic in the University Hospital and Medical Faculty of Charles University in Hradec
QUALITY OF LIFE OF LITHUANIAN CHILDREN SUFFERING FROM CANCER

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Cancer is the most often cause of death in children. According to data of the Lithuanian cancer registry, in the last decade 70–100 new cases of children cancer were diagnosed yearly. In Lithuania, the quality of life of children suffering from cancer until now has not yet been evaluated properly. Aims. The aim of the study is to increase the understanding of the quality of life of Lithuanian children suffering from cancer. Methods. The study started in February of 2005 in the Division of Oncology and Hematology at the Clinical Hospital of Kaunas University of Medicine and in the Division of Oncology and Hematology at the Vilnius University Children’s Hospital. During one year, 63 children aged 2–18-year and their families were invited to participate in the study. In the sample, 55% of children suffered from hematoblastosis, 13% from CNS tumors, and 32% from solid tumors of other localizations. The children and their families were invited to participate in the study. In the sample, 55% of children suffered from hematoblastosis, 13% from CNS tumors, and 32% from solid tumors of other localizations. The children and their families were invited to participate in the study. In the sample, 55% of children suffered from hematoblastosis, 13% from CNS tumors, and 32% from solid tumors of other localizations.

Methods. We have screened an unselected group of 20 patients, 17 men and 3 women. 16 of them had β-thalassemia major, 2 intermediate β-thalassemia and 2 α(0)-thalassemia. The mean age of the patients was 30.5 years. One of the patients presented ulcer of the lateral malleolus, another had avascular necrosis of the femoral head. A group of 20 sex- and age-matched healthy individuals served as control group. DNA analysis was performed by polymerase chain reaction and reverse hybridization. Both patients and healthy individuals were checked for 9 mutations: FV G1691A (Leiden), FH 1299R (R2), Factor XIII V34L, β-Fibrinogen 455 G-A, PAI-1 4G/5G, GPIIIa L33P (HPA-1), ACE I/D, Apo B R3500Q and Apo E2/E3/E4. Aims. The aim of the present study was to check whether the presence of a thrombophilic mutation in a thalassemic patient increases the risk for the development of a thromboembolic event.

<table>
<thead>
<tr>
<th>Table 1. Number and percentage of thrombophilic mutations.</th>
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<tr>
<td><strong>Mutation</strong></td>
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</tr>
<tr>
<td>Factor V G1691A (Leiden)</td>
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<tr>
<td>Factor V H1299R (R2)</td>
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<tr>
<td>Prothrombin G20210A</td>
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<tr>
<td>Factor XIII V34L</td>
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<tr>
<td>B-Fibrinogen 455 G-A</td>
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<td>PAI-1 4G/5G</td>
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<td>GPIIIa L33P (HPA-1)</td>
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<td>MTHFR C677T</td>
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<td>MTHFR A1298C</td>
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intermediate β-thalassemia and ulcer of the lateral malleolus was heterozygous for FXIII V34L, β-fibrinogen '455 G-A, PAI-1 4G/5G, MTHFR C677T. The 57-year old man with Sβ-thalassemia and avascular necrosis of the femoral head was heterozygous for prothrombin G20210A, FXIII V34L, B-Fibrinogen '455 G-A, PAI-1 4G/5G, MTHFR A1298C. Conclusions. The prevalence of the thrombophilic mutations in thalassemic patients doesn’t differ much of the prevalence of these mutations in non thalassemic people. However, the presentation of any of the thrombophilic mutations in a thalassemic patient is a factor that contributes, among others, to the development of a thromboembolic event.

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AUDIT OF INDICATIONS FOR OUT OF HOURS COAGULATION SCREENING AT THE ULSTER HOSPITAL

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The number of coagulation screens performed out of hours has rapidly been increasing, with a corresponding rise in the cost to laboratories, both in terms of materials and staff time. In January 2005, almost 700 coagulation screens were performed out of hours. It was felt that many of these were not clinically indicated, and most were normal. Of the abnormal results, only a small percentage were actually treated. It was therefore felt that an audit in this area was appropriate, to examine how this resource is being misused. The aims of the study were as follows: - to study the out of hours coagulation screens performed in the Ulster Hospital, to produce guidelines to be followed prior to performing the test; to rationalise the number of tests performed. 100 patients who had coagulation screens performed out of hours in January 2006 were randomly selected. The indications for the test were examined; appropriate indications included known or suspected liver disease, history of haemorrhage, current haemorrhage or renal failure. We also looked at treatment given for abnormal results. We felt that treatment should be given if the result was abnormal by 50% or more. The total number of screens performed in the one month period was 677, taken form 592 patients. Of the 100 cases we examined, 70% were in fact normal. Only 35% of the tests were clinically indicated. Only 10% of the patients who had abnormal results were treated. We concluded from the study that the majority of coagulation screens performed out of hours in the Ulster Hospital were not indicated; even if the results were abnormal, most were not acted upon. We therefore feel that local and national guidelines ought to be developed, to reduce wastage of this resource.

1490
PRE-EMPTIVE ANTIFUNGAL THERAPY IN HIGH-RISK PATIENTS WITH ACUTE LEUKEMIA: COST-EFFECTIVENESS OF INTRAVENOUS ITRACONAZOLE

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Backgrounds. Systemic fungal infection remain a major clinical problem in immunocompromised patients, particularly in patients with prolonged severe neutropenia, preexisting myelodysplasia and advanced age. In these cases, presumed systemic fungal infections are treated empirically to reduce documented infections and associated mortality. Aims. We, retrospectively, compared the cost-effectiveness of intravenous itraconazole treatment with conventional treatment with lipo-somal amphotericin-B or new antifungal drug as voriconazole, in patients affected by AML or high-risk MDS. Methods. Since January 2005 to December 2005, 88 patients (24 female and 14 male, median age 72) affected by AML or high-risk MDS, who underwent induction chemotherapy, received primary antifungal prophylaxis. Twenty patients were treated with fluconazole 200 mg/day os and 18 patients with itraconazole 200 mg bid os. During induction therapy the median length of severe neutropenia (PMN<500/m3) was 19 days. Fever episodes have been empirically treated with broad-spectrum antibiotics (cephalosporine plus aminoglicoside with or without glycopeptide). Pre-emptive and empirical antifungal treatment for fever unresponsive to broad-spectrum antibiotic therapy was employed, after 5 days, in 20 fluconazole patients with liposomal amphotericin-B 3 mg/kg/die intravenous in 9 patients or voriconazole 6 mg/kg bid during first 24 day followed by 4 mg/kg bid intravenous in 11 patients. In the subgroup treated with oral itraconazole all patients were switched to intravenous drugs at the dose of 200 mg over 60 minutes every 12 hours during the first 2 days followed by 200 mg given i.v. once daily. All patients were treated with pre-emptive therapy for a median of 14 days (11-21). Results. There were no significant differences noted between the three subgroups with regard to the duration of prophylaxis (median: 10 days for fluconazole vs 11 days for oral itraconazole), percentage of patients who developed fever unresponsive to broad-spectrum antibiotic therapy (46% in fluconazole group vs 39% in itraconazole group), proven/probable or possible fungal infection as well as with regard to survival. Safety and toxicity analysis of pre-emptive treatment was similar in all subgroup, only one patients withdrawal from voriconazole therapy for hallucination and one withdrawal from intravenous itraconazole for nausea and vomiting therapy resistant. When we compared cost of three pre-emptive therapy we showed that lipid amphotericin-B and voriconazole were most expensive than intravenous itraconazole both we consider daily treatment cost and total treatment associated with nurse cost. Conclusion: Intravenous itraconazole has at least equivalent efficacy as empirical antifungal therapy in immunocompromised patient affected by AML or high-risk MDS. However, intravenous itraconazole compared with other antifungal treatment was shown to be the best cost-effective and cost-saving pre-emptive empirical therapy.